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PIROPLASMOSIS.

(1) NUTTALL (G. H. F.) & STRICKLAND (C.). **On the Occurrence of Two Species of Parasites in Equine "Piroplasmosis" or "Biliary Fever."**—*Parasitology.* 1912. Feb. Vol 5. No. 1. pp. 65–96. With 1 plate, 1 text figure, and 5 charts.

According to these authors there are included under the term *Piroplasma equi* two separate and distinct parasites, and consequently two distinct diseases have hitherto been included under the name piroplasmosis.

In their view the true piroplasms always multiply in a characteristic manner, the peculiar piriform parasites developing by a process resembling budding from large rounded or slightly amoeboid parasites, the whole of whose protoplasm flows into the buds and gives rise to two piriform parasites without any residual body being left.

The description of the *Piroplasma equi* given by LAVERAN, who examined stained specimens sent by THEILER (1901), does not indicate this as the typical method of multiplication. FRANÇA convinced of the difference between Laveran's parasite and the Piroplasma, has placed the parasite in a new genus, named *Nuttallia.*

These parasites do not multiply according to the method described for Piroplasma; they do not occur as pairs of piriform parasites, and they form distinctive "cross-forms" which BOWHILL and FRANÇA regard as multiplication forms.

The occurrence of two types of parasites in equine piroplasmosis was pointed out by KOCH (1905), who believed that they might cause distinct diseases.

A short preliminary note was published upon this subject by the present authors in 1910, in which they showed that two distinct parasites occur in horses suffering from biliary fever, and consequently two distinct diseases are included under the name.

A strain of the *N. equi* was obtained from MCFADYEAN, the original having been obtained from South Africa and the strain maintained by passage through a number of horses. The blood was taken from a horse that had been inoculated with positive results two years previously.

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To avoid confusion the authors first describe their observations on the *N. equi*, subsequently dealing with the true Piroplasma (*P. caballi* Nuttall 1910), which also occurs in horses suffering from biliary fever.

Nuttallia Equi.

Horse 1.—Inoculated with blood obtained from McFADYEAN. Parasites appeared on the 8th day and the horse died on the 11th day.

Horse 2.—Inoculated with blood from Horse 1 on the day of its death. Parasites appeared on the 7th day and disappeared on the 22nd day. Horse recovered and still alive (after more than 2 years).

Horse 3.—Inoculated with blood from Horse 2 about seven months after the disappearance of the parasites. Parasites appeared on the 10th day and the horse died on the 20th day.

The living parasite.—This was studied in fresh blood films at body temperature.

The number of infected corpuscles was small. In Horse 1 it was 5·4 per cent.; in Horse 3, 13·2 per cent. In Horse 2 the parasites appeared in the blood in such small numbers that there was no adequate opportunity of counting them. The following types of parasites were studied:—

Small parasites.—Measured 1-1·4 microns, frequently amoeboid, moving about within the corpuscle while altering their shape. Occasionally, filiform or bud-like processes were protruded and retracted. The escape from corpuscles was never observed, nor were any of the small parasites observed to divide.

Single medium-sized parasites behaved in much the same way.

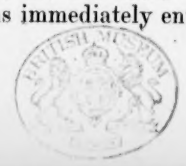
Very large single parasites, of which a certain number may be found, may show very little change of form, but they may assume an elongated or plump piriform shape.

Dividing forms.—In a number of cases stages of development of a single parasite into four were observed. In one case a previously rounded parasite was observed to give rise to four parasites after developing into a cross-form. In the majority of cases the cross-forms break up into four distinct parasites which wander away from each other, but in a very small number of cases five and six small parasites were observed to result from the breaking-up of a cross-form. The resulting young parasites are very active.

In a few cases multiplication occurred by a process of budding, but such appearances may be deceptive, for when an infected corpuscle ruptures, the main mass of the protoplasm may be seen to be connected with the supposed daughter individuals by delicate strands of protoplasm.

The escape of the young parasites resulting from the breaking-up of cross-forms was observed to occur in some instances soon after their separation from each other. The corpuscle was not ruptured in the process. The escaped parasites after swimming about vigorously for a few minutes disappeared, the conditions *in vitro* being doubtless unfavourable to their continued existence.

Although the authors are convinced that the young parasites under natural conditions immediately enter a new corpuscle, such



an entry was not actually observed. On one occasion a medium-sized parasite was observed to leave a corpuscle and degenerate without injuring the corpuscle. It was noted several times that the first parasites to escape were the small ones resulting from the breaking-up of cross-forms. Some of the corpuscles vanished while the contained parasite remained motionless, and in other cases the corpuscles burst suddenly and the parasites were ejected into the plasma.

Stained parasites.—The parasites stain in a manner similar to Piroplasma. About 90 per cent. of the invaded corpuscles contained single parasites of various shapes and sizes. About 2·5 per cent. contained 2-4, and 1·5 per cent. dividing or cross-forms. The number of free parasites encountered differs widely. In Horse 3 only a very small percentage of free forms were found (0·2-6 per cent.) and in Horse 1 as many as 26 per cent.

A diagram is given showing the "usual mode of multiplication of the *Nuttallia equi* in the circulating blood." The minute piriform parasite enters a fresh corpuscle and increases in size, is slightly amoeboid, with a general tendency to assume a pear-shape. When the parasite has attained a certain size, definitely amoeboid movements are observed. Judging from the form of the chromatin in stained specimens the next steps are as follows. The chromatin changes its shape from round to elongated and lies at the periphery of the parasite in the form of a curved rod. This then assumes a V-shape, small thickenings appearing at the free and the united ends of the arms of the V. The arms themselves disappear leaving the knob-like thickenings only, which become separated at equal distances from each other. The cytoplasm then shows a tendency to be pinched off into the form of a cross, one of the chromatin masses being in each arm. These stages, from the separation of the four little chromatin masses to the formation of the cross, have been observed in moist preparations.

Distribution of the parasites in the body.—The parasites appeared to be fairly uniformly distributed in all the organs examined.

In the case of Horse 3, which had been previously infected with *P. caballi* (see later), there were roundly 9 million red corpuscles per c.mm. at the time when parasites appeared, and by the 19th day this number had fallen to 3 million. Differential counts were made on four days, and it was found that there was a marked decrease in the number of eosinophiles.

Piroplasma (Babesia) caballi.

Horse 1.—Inoculated with blood obtained from Marzinowsky. Parasites appeared on the 15th day. Horse died on the 22nd day.

Horse 2.—Inoculated from Horse 1. Parasites appeared on the 8th day. Horse recovered. This animal was subsequently inoculated with the *N. equi* and is No. 3 of that series.

Horse 3.—Parasites appeared on the 9th day. Horse died on the 19th.

The living parasite.—Although the scarcity of the parasites in the blood made it impossible to make a complete study of the

development *in vivo*, the authors are convinced that *P. caballi* develops in a manner similar to the *P. bovis* and *P. canis*.

The forms of parasites seen correspond exactly with those observed in *P. bovis* and *P. canis*, and the process is slower than in the case of the latter.

In stained preparations the parasite appears to be very closely allied to *P. bovis*.

The predominant forms found in stained preparations were the rounded and double piriforms.

In Horse 3 there was a relatively slight decrease in the number of red corpuscles and a slight fall in the amount of haemoglobin. The leucocytes showed a decided decrease as the disease advanced, accompanied by a disappearance of eosinophiles and an increase in neutrophiles.

Immunity test with regard to N. equi *and* P. caballi.—As already mentioned Horse 3 of the *N. equi* series is the same animal as Horse 2 of the *P. caballi* series.

It was first inoculated with *P. caballi*, suffered from a mild infection, and to all appearances made a good recovery.

Two months later it was inoculated with *N. equi* and died of " biliary fever " 20 days later. Only *N. equi* could be found in the blood. This was the only horse of the six that had haemoglobinuria, and it is possible that the mixed infection was responsible for this.

Conclusions.—

The term " Biliary Fever " or " Piroplasmosis," hitherto supposed to apply to a specific disease affecting horses, in reality refers to two distinct diseases produced by distinct parasites. For convenience' sake, and in accordance with the terminology at present in vogue, these two diseases may be named after the parasites which produce them, *i.e.*, Piroplasmosis (due to *Piroplasma* [or *Babesia*] *caballi* Nuttall, 1910) and Nuttalliosis (due to *Nuttallia equi* (Laveran, 1910, França, 1909).

(2) Schuberg & Reichenow. **Über Bau und Vermehrung von Babesia canis im Blute des Hundes.** [The Morphology and Multiplication of *Babesia canis* in the Blood of the Dog.] *Arbeit. a. d. Kais. Gesundhtsamt.* 1912. March. Vol. 38. No. 4. pp. 415–434.

The authors employed principally Schaudinn's method of moist fixation followed by staining with Giemsa's solution. Examinations were also made of the living parasite on the warm stage.

The strain used at the commencement of the experiments was a very virulent one, young dogs dying in 3-4 days after infection. Owing to circumstances the experiments had to be interrupted and it was found that the strain in the infected dogs had lost much of its virulence and that it was very difficult to re-instate this.

The small number of parasites present in the blood of some of the dogs enabled the authors to investigate their distribution in the body. Large masses of parasites were found in the kidneys and also in the capillaries of the lungs. There is a striking difference between the number of parasites in the peripheral capillaries and the large vessels, and the authors state that, if at

the onset of the temperature reaction a small cut be made in the ear, the first drop of blood which escapes may be very rich in parasites, while the second may be so poor that prolonged search is required to find even single ones.

In fresh preparations of such blood there may frequently be observed masses of blood corpuscles which are adherent to each other. The majority of such corpuscles may be invaded with Babesia. This is explained by the fact that in cases of piroplasmosis the corpuscles have a tendency to cling together, and that this causes plugging of the capillaries and the multiplying parasites naturally invade the cells nearest at hand.

Agglutination of red cells begins before the parasites make their appearance in the circulation, and it may occur as early as 48 hours before the blood invasion. The authors found in one case at least that although there was obvious agglutination of the red corpuscles in blood taken from the ear, it was not discoverable in the heart blood when the animal was killed soon after.

Canine piroplasmosis is associated with a marked increase in the number of mononuclear leucocytes, and these frequently contain not free parasites, but entire invaded red corpuscles.

Morphological changes observed during multiplication.—The authors agree with NUTTALL and GRAHAM SMITH that the pairs of pear-shaped parasites are formed by a process of budding from rounded parasites, the buds growing at the expense of the mother-cell. They believe however that the rounded cell which gives rise to the daughter cells is not intra-corpuscular. They hold the view that the amoeboid cells which precede the rounded parasites exhibit movements that are too active to permit of the view that the parasites are intra-corpuscular. Comparison is drawn between these and the slow movements exhibited by the merozoites of tertian malaria which are intra-corpuscular.

The parasite regains the interior of the red corpuscle according to the authors by means of the buds which develop into the pear-shaped parasites. These are described as boring into the corpuscle and there developing at the expense of the mother cell outside. The parasites present in multiple infection of a red cell are almost always developed from a single parasite as is evidenced by the fact that the parasites are all at the same stage; the authors have however occasionally observed multiple infection with the parasites in different stages. The pair of parasites developed from the first amoeboid parasite again pass to the outside of the cell, become amoeboid, and penetrating again by means of their processes double their infection, and so on. The authors have never observed more than 16 parasites in a single corpuscle. They have, further, never observed the simultaneous formation of four pear-shaped parasites as described by NUTTALL and SMITH, and CHRISTOPHERS.

Cytology.—The cytoplasm of the Babesia is finely alveolar in structure. In practically every parasite one or two clearer portions can be seen. In dry preparations these appear like vacuoles, but in preparations that have been fixed moist fine strands of protoplasm are observed to traverse the apparent spaces. These " vacuoles " are certainly not part of the nuclear structure, since in many parasites the nuclear substance is quite outside them.

In the majority of the pear-shaped and amoeboid parasites the nucleus is in the form of a single, round, homogeneous mass of chromatin which possibly contains a minute paler portion. When an amoeboid parasite is about to divide the mass of chromatin becomes elongated, one pole appearing to be richer in chromatin than the other. The two poles then separate from each other and finally two rounded masses of chromatin are formed. Each again divides and while this is in progress the buds begin to form, one portion of the chromatin going into each bud and then a second portion. In this way the two pear-shaped parasites are each provided with two pieces of chromatin. The difference in texture between the two pieces is now very obvious, one being large and the other paler and smaller. When the twin parasites separate this appearance of two portions of chromatin is lost. Delafield's haematoxylin is superior to Giemsa's stain for showing the changes in the chromatin.

The pear-shaped parasites which have been set free by the disintegration of their host cell appear in fixed preparations to be shorter than the intra-corpuscular forms. They also appear to be more intensely stained; a possible explanation of this being that the intracellular forms are flattened and consequently thinner. In the majority of cases the free parasites contain only one piece of chromatin.

The authors do not agree with the statements made by other investigators regarding the occurrence of flagellated forms.

Brief criticisms are given of the descriptions by various authors of the multiplication of the parasite in culture, and of the developmental stages in the tick, but nothing new is added.

The authors do not consider the second mass of chromatin to be representative of a blepharoplast but rather of a nucleolus, and they think that in the present state of knowledge Babesia should be considered to be more closely allied to the Coccidia than to the Binucleata (Hartmann).

(3) Ciuca (A.) **Recherches sur l'Influence de la Splénectomie totale sur l'Evolution de la Piroplasmose canine.** [The Effect of Total Splenectomy upon the Evolution of Canine Piroplasmosis.]—*Bull. Soc. Path. Exot.*, 1912. Feb. Vol. 5. No. 2, pp. 143–150.

After a brief reference to the varying opinions held by different authors regarding the effect of splenectomy in different diseases the author describes his experiments in connection with canine piroplasmosis.

His experiments were grouped into three classes:—

A. Those in which the spleen was removed from normal dogs which were subsequently infected.

B. Those in which the dogs were infected before excision of the spleen.

C. Those in which the spleen was excised when the piroplasmosis was at its height.

The conclusions drawn are as follows:—

1. Infection subsequent to removal of the spleen, provided the dog has completely recovered from the operation, does not affect the course of the disease, setting aside complications.

2. Splenectomy during the evolution of the disease undoubtedly aggravates the symptoms, and especially in young animals. This is the more evident in cases in which there is some debilitating condition as a complication of the disorder caused by the splenectomy.

3. The reappearance of the parasites and the aggravation of the symptoms are less marked in dogs that are operated upon from 3-12 days after the disappearance from the blood of the last infected corpuscles.

4. When the animal has passed through an attack and is on the road to recovery splenectomy in the great majority of cases does not lead to any reappearance of the parasites. In exceptional cases there is a slight and transient reappearance.

5. In dogs that are operated upon immediately after the disappearance of the parasites, and in those operated upon during the course of the disease there is often an immediate and very marked hyperleucocytosis, which steadily increases up to the death of the animal.

In all the experiments, and especially in those in which the animals were infected after splenectomy, one must hesitate before accounting for the conditions observed by the absence of the spleen. Operative shock and accidental infections appear to play a more important part than the absence of the spleen.

A practical conclusion may be drawn that splenectomy is not a practicable method of preserving the virus, and all the more in that even in animals that have not been operated upon the blood is always infective for some months and even a year without any parasites being visible in it.

(4) Inchiostri (H.). **Vorkommen und Formen der *Piroplasma ovis* in Dalmatien.** [Piroplasmosis of the Sheep in Dalmatia.]—*Oesterreichische Wochenschr. f. Tierheilkunde.* 1912. Vol. 37. No. 29, pp. 289–292; No. 30, pp. 299–302; No. 31, pp. 310–313; No. 32, pp. 320–322; No. 33, pp. 331–332; No. 34, pp. 340–343.

The author's investigations were carried out in the Zara District of Dalmatia. The typical piroplasms were found in smears of peripheral blood stained with various modifications of the Romanowsky method. Multiple infection of a single corpuscle occurs, as many as 8 parasites having been observed. Exact methods of measuring the parasite were not available, but taking the size of a red corpuscle as 4-6 microns the author judged that the parasites vary in size from ½-3 microns. Both intra- and extracorpuscular forms were encountered. During life the parasites are more numerous in the peripheral blood than in the large vessels. Piroplasms occur in large numbers in the spleen.

In moist preparations the smallest of the parasites and those that are undergoing division show very active movements. As a rule, the intracellular parasites remain in the same corpuscle until it ruptures, but occasionally piroplasms have been observed to leave corpuscles without causing their destruction. Such liberated parasites actively seek out another red cell, and penetrate it by

means of a pointed projection which is suddenly formed. Repeated attempts to penetrate different corpuscles have been observed.

Parasites have been observed in the interior of leucocytes as a result of phagocytosis.

In moist and stained preparations large round parasites have been observed which are provided with a long pseudopodium. Parasites of this type are rarely encountered within red cells, but are as a rule extraglobular.

The *Rhipicephalus bursa* is the sheep tick that occurs in the district, and in blood in these ticks the author has found the star-shaped parasites described by Dschunkowsky and Luhs. In addition to these he has observed immense numbers of small rounded parasites which execute amoeboid movements. Only ring- and star-shaped parasites were observed in the fully engorged females, the small round forms occurring exclusively in the larvae and nymphs.

The predominance of pear-shaped parasites in stained preparations may be explained by supposing that contraction has occurred during the process of fixation.

The author found that the parasites in the spleen had lost all power of movement within 12 hours of the death of the host, but by the addition of salt solution the motility was restored and the parasites made to retain their vitality for a long time. Parasites are scarcely recognisable in the internal organs within 24 hours after death, and shortly after this they have entirely disappeared.

Parasites present in the blood have been proved to be living 8-10 days after the death of the host.

The author describes the occurrence of peracute, acute, abortive, and chronic cases of the disease, and gives a detailed account of the symptoms observed in each variety.

The peracute form occurs principally during the hot season of the year and almost exclusively attacks ewes that are in poor condition. The temperature rises suddenly and may reach 42° C. or more. Before death, which occurs very shortly, the temperature falls again, and is almost invariably subnormal at the time of death.

Jaundice is a very constant symptom. There are frequently observed symptoms of colic, and rigors are prominent. There is always haemoglobinuria in the peracute cases.

The following lesions are found: Haemorrhages and gelatinous infiltration in the connective tissues; petechiae in the muscular masses; jaundice; hyperaemia and oedema of the brain with haemorrhages in the dura and spinal membranes; swelling and infiltration of the red marrow of the long bones. There is always enlargement of the spleen, but not to the extent observed in anthrax. The spleen pulp is never fluid and the author has observed only one case of rupture of that organ.

In the acute form of the disease the duration of illness is longer, the temperature does not rise so high and some cases recover. Haemoglobinuria does not persist for very long; in many cases it ceases after a few hours. Frequently there is blood-stained diarrhœa.

Acute cases last from 3-5 days, and in those in which the animals are going to recover the acute symptoms abate in 10-12 days. Convalescence is very slow. There is often complete loss of fleece in animals that survive an acute attack. Relapses are not uncommon.

A mild form of the disease occurs in the spring and autumn. It is rarely seen in adult animals, but principally affects the lambs.

An experiment was carried out with the object of testing the difference in the resistance offered to the disease by strong and by weakly animals, the inoculation material being virulent blood and blood from a recovered animal. The resistance offered by indigenous sheep as against that offered by other sheep was also tested.

Five animals in good condition and five weakly ones were inoculated with blood from an immune animal, with the result that all the five healthy animals recovered from the disease so induced and two of the weakly animals died. A test carried out with fully virulent blood (blood containing numerous parasites) had similar results.

Three sheep from Croatia inoculated with immune blood all succumbed.

In order to ascertain for what period animals remained infective after an attack of the disease, a number of animals were inoculated with blood from immune animals at intervals varying from 2-11½ months after the recovery of the immune animals from the attack which conferred immunity upon them. The dose in each case was 10 cc.

Only those inoculated at an interval of 2 months died. Those inoculated at 3½ and 5 months suffered from a mild attack but recovered, and those inoculated subsequently did not react. The blood used for those animals that died contained piroplasms, but in that used for subsequent animals none could be found. The author has been able to demonstrate piroplasms in the blood for periods varying from 2 to 12 months after recovery. Those cases in which the parasites persist for a long time he terms " latent " cases.

Chronic cases are those in which there are frequent slight relapses, and the course of such cases closely resembles that followed by cases of infection with liver fluke, the affected animals gradually wasting away and becoming generally cachectic.

A number of drugs were tried by the author in the treatment of the disease, but none gave constantly satisfactory results.

ANAPLASMOSIS.

(5) **Theiler (A.). Weitere Untersuchungen über die Anaplasmosis der Rinder und deren Schutzimpfung.** [Further Investigations regarding Anaplasmosis and Protective Inoculation against the Disease.]—*Zeitschr. f. Infektionskrankh., parasit. Krankh., u. Hyg. d. Haust.* 1912. March 27. Vol. 11. Nos. 3–4, pp. 193–207.

Having given a brief resumé of his reasons for believing Anaplasma to be a distinct species of parasite, the author proceeds to describe experiments in support of this view.

1. The Separation of Anaplasmosis from Babesiosis by proper Selection of Ticks.—

As is well known an animal that has passed through an attack of either Texas fever or anaplasmosis, although immune, is a carrier of parasites, which may be transmitted to susceptible animals either by ticks or by inoculation with blood. If an imported English animal be inoculated with blood from an animal that has been bred in a tick infested district, one or other, or as a rule both, of the diseases may be expected to develop. For a number of years the author has observed that calves which he has obtained from the Karoo, which is only lightly infested with certain species of ticks, have shewn a striking degree of susceptibility to redwater and only a slight degree to anaplasmosis. This aroused the suspicion that these calves were possibly immune to anaplasmosis, the animals having passed through an attack when quite young, and that the animals acted as carriers of the parasite.

This view received some support from the fact that the blue tick (*Rhipicephalus decoloratus*) was unknown in the district, the black-pitted tick (*R. simus*), which together with the blue tick is a host of the Anaplasma, alone being found. Calves were obtained from two Karoo farms. Experiments were made on calves a few weeks old born on the premises of the laboratory, in order to ascertain whether the *Babesia bigeminum* could really be excluded.

Three young calves were inoculated with blood from the same number of Karoo calves with the result that two shewed a double infection and the third a pure anaplasma infection. The Karoo calf from which the anaplasma (pure) infection was derived was obtained from a different farm to the others. The experimental calf was subsequently inoculated with immune babesia blood and developed a typical attack of Texas fever, the parasites being fairly numerous in the blood. This calf was used as the starting point of a series of inoculations into susceptible English animals.

Twelve English animals and one Africander calf were inoculated, the dose varying from 1-100 cc. In no single case was there any infection with the *Babesia bigeminum*, shewing that a pure infection with the Anaplasma occurs naturally.

Some of these animals were afterwards tested with a view to ascertaining whether they were immune to redwater. Six were inoculated with blood, and three by means of ticks. With one exception, in which case the reaction was of a doubtful nature, all the animals reacted typically, and in some instances the reactions were so severe that treatment with trypanblue had to be resorted to.

A striking feature of the anaplasmosis infections was that none of the animals died, and that the disease ran a mild course in practically every case. The majority of the cases would have escaped observation if the thermometer and the microscope had not been used. This result was in striking contrast to results obtained with English animals in earlier experiments, and indicated a great variation of virulence. On microscopic examination it was found that the Anaplasma used in these experiments possessed certain characters by which it could be distinguished from the Anaplasma previously found.

Whereas the parasite first found was in the great majority of cases disposed towards the rim of the invaded red cell, and only exceptionally in the centre, the parasite observed in the series of experiments under consideration was most frequently found near the centre of the invaded blood corpuscle.

Apart from this there was a difference in size between the parasites. The difference could not be expressed exactly in figures, but was quite apparent to the skilled eye; those disposed at the margin of the corpuscles being generally larger than those at the centre. On these grounds the author concludes that there are two varieties of Anaplasma, and designates the form that is found centrally placed in the corpuscle as *Anaplasma marginale* (var. *centrale*).

2. Testing of Animals immune to *Anaplasma centrale* with *Anaplasma marginale*.—

Six animals that had recovered from red water and central anaplasmosis were inoculated with the *A. marginale* with the result that five reacted after the usual period, although the attacks were mild.

This shews that *A. centrale* protects against the *A. marginale* to the extent of preventing a severe attack of the disease, but the immunity is not complete. From a theoretical point of view therefore, the parasites, which are distinguishable from their position in the corpuscles and their difference in virulence, should be considered as varieties of one and the same species.

Notice should be taken of the fact that every animal whether suffering from Texas fever or anaplasmosis developed mutans piroplasmosis when injected with the *Babesia mutans*. This is further evidence of the specific nature of this parasite.

3. Protective Inoculation against Anaplasmosis.—

It having been shewn that the *Anaplasma marginale* var. *centrale* was the cause of a mild illness from which all the experimental animals recovered, and that such animals possessed a considerable degree of immunity against the *A. marginale* infection, the question naturally arose as to whether the *A. centrale* could be used as a protective inoculation against natural infection. Owing to the susceptibility of imported animals to the two diseases —redwater and gall-sickness—the question had to be decided whether the animals should first be immunised against redwater, controlling the inoculation with trypanblue if necessary, and then against the Anaplasma; whether the protective inoculations should be carried out in the reverse order; or whether the two should be carried out together.

(*a*) Inoculation against redwater followed immediately by inoculation against anaplasmosis.

Three animals were inoculated with anaplasma blood after the subsidence of a reaction due to previous inoculation with redwater. In all three cases reactions occurred at the end of the fourth week and were very mild, the maximum percentage of invaded blood corpuscles being 8·6.

(*b*) Inoculation with Anaplasma followed after the subsidence of the reaction by inoculation with redwater.

Three animals were also used for this experiment. In each case the attack of anaplasmosis was slight, but the redwater caused by the subsequent inoculation with redwater blood on the 40th day was in one case so severe that resort was had to trypanblue; the other reactions were mild.

Three further animals were used for an experiment of the same nature. In the first animal there was a slight anaplasmosis reaction and redwater blood was inoculated on the 40th day. The resulting reaction was so severe that trypanblue was used. The two other animals developed very mild attacks of anaplasmosis but failed to react to the redwater inoculation, a *Babesia bigeminum* infection having developed in both the animals during the incubation period of the anaplasma infection. This anomalous result might be explained either by the animals injected having contracted redwater infection naturally on the veldt, the anaplasmosis inoculation causing a relapse, or the animal from which the anaplasmosis infection was derived might in some way have become infected with the *Babesia bigeminum*. The striking fact remains that the whole of the animals reacted. The second suggested explanation appears to be the more probable in view of the fact that the animal which supplied the blood for the anaplasmosis inoculations was found to be refractory to a redwater inoculation.

(*c*) Simultaneous inoculation with redwater and anaplasmosis.

Five animals received simultaneously doses of blood containing the *A. centrale* and *B. bigeminum*, the doses varying from 5-50 cc. The results shewed that the procedure was without danger provided that the Babesia infection was controlled by the use of trypanblue. From a practical point of view an answer was required to a further question, whether animals so immunised could be immediately exposed to natural infection.

In view of the shorter periods of incubation in experimental cases than in natural cases of infection one might suppose that animals protected experimentally would resist natural infection.

Animals that had been inoculated with African redwater in England were inoculated with anaplasma immune blood after their arrival in Africa, and had placed on them while in the stalls a large number of infected ticks. After the subsidence of the reaction they were turned out. The object of placing the ticks upon them was to set up a natural attack within the period of incubation.

Five animals were used and in none of them was there any reaction that was clinically recognisable. Possibly the animals reacted to the Anaplasma after a long period of incubation, but as no clinical symptoms were observed further blood examinations were not carried out. Cattle that are immune to redwater may be exposed to natural infection with *Babesia bigeminum* during the process of immunisation against Anaplasma without running any risk.

Conclusions.—At least two forms of anaplasmosis can be distinguished—a virulent and a less virulent. The diseases can be transmitted by inoculation with immune blood without any exaltation of virulence. Recovery from the mild form of the disease

protects against the severe form, but there is no complete immunity. The two diseases are recognisable microscopically from the size and position of the causal parasites in the red corpuscles. In the severe form the parasite is generally disposed towards the rim of the invaded cell (*Anaplasma marginale*) and in the mild form towards the centre (*Anaplasma marginale* var. *centrale*). The peripherally placed parasites appear to be larger than those that are centrally placed. This fact is of importance in connection with protective inoculation, and the results obtained in the field confirm the accuracy of this conclusion. As far as South Africa and indeed the greater part of Africa is concerned, protection must be conferred upon imported animals from Europe if the trade is to flourish. This is without danger if pure *Babesia bigeminum* be used for the inoculation; that is to say, the virulent *Anaplasma marginale* must not be injected.

By means of methodical inoculations this pure strain can be maintained, as can a pure strain of the *Anaplasma centrale*. In the Laboratories of the Union of South Africa at Onderstepoort and Grahamstown both these strains were maintained in a state of purity.

(6) Basile (C.). **Sull'Anaplasma canis.** [Canine Anaplasmosis.]—*Pathologica.* 1912. June 15. Vol. 4. No. 87. pp. 358–360.

During the course of his examinations of the blood of a number of different species of animals in the country around Messina, the author encountered a parasite in the blood of a dog which was very like Anaplasma. The parasite was observed in smears made from the peripheral blood of an adult dog which was in a very anaemic and poor condition. The smears were fixed and stained with Giemsa.

An attempt was made to transmit the disease experimentally. With this object blood was drawn with aseptic precautions from the liver of the infected dog and injected intraperitoneally into a puppy about a month old. This animal was protected from blood-sucking insects, and its blood was frequently examined for parasites before the experiment was commenced without any being found. Another puppy of the same litter was kept as control, and its blood was examined for parasites with negative results.

A few days later the infected dog was killed in order to study the distribution of the organism in its body. The parasites were found to be very scanty in the spleen, scanty in the liver and numerous in the lungs.

The inoculated dog appeared to be perfectly healthy until the tenth day after inoculation. Anaplasma was found in smears of the hepatic and peripheral blood stained with Giemsa.

Before the experimentally infected dog was killed blood was drawn from the liver with aseptic precautions and injected immediately into the peritoneal cavity of a second puppy, another of the same litter being kept as control. The blood of this dog was also examined, but no parasites were found.

At the post-mortem of the first experimentally infected dog parasites were found to be scanty in the spleen and numerous in the liver.

The second experimental dog refused all food after 24 hours and died on the third day. The course of the disease was very acute, and parasites were found in the blood of the peripheral vessels, spleen and liver.

The control dogs were killed when they were in perfect health and no abnormalities were found at the post-mortem.

Morphology of the Parasite.—The author has observed two forms of the parasite: one a coccus-like body which is apparently devoid of cytoplasm, and the other a crescent-shaped body which possesses cytoplasm.

The coccus-like bodies are rounded or oval in shape and measure about 2 microns. They are found in smears of blood from peripheral vessels, liver, spleen and lungs. They may be free or enclosed in corpuscles. The intracorpuscular forms are either centrally or eccentrically placed. Double, treble, and quadruple forms have been observed, and the author believes this multiplicity stands in some relation to the method of multiplication of the parasite.

The diplococcus form sometimes presents a dumb-bell-like appearance. In preparations that are not intensely stained it is possible to distinguish a marginal portion stained an intense rose-violet surrounded by a clear halo. In the interior of the parasite may be observed a number of granules of chromatin more intensely stained.

The crescent form of the parasite was found only in the dog naturally infected and in the second of the experimental animals.

The semilunar bodies vary from 3-5·5 microns in length. The ends may be either rounded or pointed. They consist of a body and nucleus, the body being surrounded by a peripheral zone in the form of a membrane.

The peripheral zone is not constant, but when present it stains a pale pink, and in the author's preparations was only to be seen in those parasites in which the body was stained pale blue and had a granular appearance.

The nucleus, which measures from 1-2 microns, is either central or a little towards one end of the parasite. It sometimes shews a more or less centrally-placed vacuole. In some of the parasites the nucleus is elongated and in some it is double. Within the nucleus there is a granule of chromatin that is more intensely stained, and in some cases there can be seen another granule of chromatin either close to or a little removed from the nucleus.

In a preparation from the hepatic blood of the naturally infected dog the author observed a single parasite which appeared to be provided with a flagellum about 5 microns in length with a thickening at its free end. The body of this parasite measured 4·9 by 2·3 microns. The cytoplasm was vacuolated and contained a large nucleus.

The author considers it premature to attempt to give any definite interpretation of the forms of the parasite found.

TRYPANOSOMIASIS.

(7) Leese (A. S.). **Biting Flies and Surra.**—*Jl. Trop. Veter. Science.* 1912. Jan. Vol. 7. No. 1, pp. 19–32.

The author's object is to show that the doubts cast on the theory of the mechanical transmission of surra, in view of Kleine's experiments with the *T. brucei* and the *G. palpalis*, are not justified. In support of his view that the *T. evansi* is commonly transmitted mechanically the author gives accounts of oubreaks of surra occurring in desert places where Tabanus is unknown.

In the monsoon season of 1908 and 1909, when the rainfall was excessive, there was an outbreak of surra in the Imperial Service Camel Corps. There were in all about 500 camels, and by January 1910 more than half of these were dead, and more than half of the remainder were affected. On tracing the history of these animals it was found that none of them had been out of the State (Bikanir) for duty and that only 40 of them had grazed outside the State in the monsoon season. All the animals save five had been sufficiently long in the service to prove that they could not have been affected at the time of purchase. The five were almost certainly diseased at the time of purchase. This obviated all difficulty of accounting for the source of the disease.

The author was able to prove that the outbreak occurred when the animals were grazing on a desert portion of the State at least 100 miles from the fly zones. Further, the State breeding herds and the animals belonging to the villagers also suffered, there being among the latter young animals that had never been beyond their village allotment. The only biting fly found was the *Lyperosia minuta*.

The second outbreak occurred among the camels kept for civil purposes in the same State during the monsoon season of 1910. The author had examined these animals in the previous January and had found them all healthy. The total number of camels was 44 and by the end of November 11 had died, and of the remainder 16 were affected. These animals had been kept together and had not been elsewhere than in or near Bikanir City and in Gajner, where there is an isolated garden belonging to the State with a tank in it, and in the desert between these two places, a distance of 20 miles.

Investigation showed that Tabanus is not found in these places, but that Stomoxys is present in both, and Lyperosia is very abundant; mosquitoes also occur in both places.

The inference drawn by the author from the facts brought to light by his investigation is that in these cases the Lyperosia was the principal agent involved in the spread of the disease.

This Lyperosia occurred in swarms on every camel, and set up intense irritation causing the animals to rub against each other. Possibly inoculation sometimes occurs in this way. The flies show no great tendency to pass quickly from one animal to another, but this must often take place as they are found in such enormous numbers and the hosts in these cases were grazed close together.

It was impossible to carry out experiments in connection with the mechanical transmission of the disease by Lyperosia during 1911 owing to the drought.

The presence of this fly is not essential to the spread of surra. In Sohawa, where equine surra was previously unknown, a single case occurred in a mare that stood about 25 yards from the camel camp. Tabanus, Stomoxys and mosquitoes were all found in this district. The author draws the following conclusions from his investigations:—

1. That surra can spread widely in the absence of Tabanidae (but in the presence of Lyperosia).
2. That surra can spread easily in the absence of Stomoxys and mosquitoes (but in the presence of Lyperosia).
3. That surra can spread in the absence of Lyperosia (but in the presence of Tabanids, Stomoxys and Mosquitoes).
4. That if *T. evansi* undergoes any life cycle in any of these biting flies, then *either* surra can spread so easily in the absence of the specific fly, if there are enough biting flies of other genera to do it mechanically, that the question of life-cycle loses its practical importance, *or*, that *T. evansi* is capable of developing in the bodies of several distinct genera of biting fly. Is it not far more likely that the common power of transmission is a mechanical one?

The author then criticises briefly the writings of a number of authors regarding the method of transmission of surra by the agency of flies and adds some notes on the occurrence of biting flies in the North-Western camel country of India.

All the biting flies except the Hippoboscids require moisture in varying degrees before they become numerous. In the Punjab there are two fly seasons, one from March to April, the other from July to October. It is the latter that is generally called the surra season, since equine outbreaks are then generally met with.

The biting flies are not evenly distributed. Tabanids are always the most numerous in the following places:—

1. The banks of rivers or nullahs which overflow at certain seasons.
2. Dense jungles in the hills where the rains are heavy.

Tabanus has a very wide distribution, but Stomoxys has a still wider. It requires less moisture than Tabanus, and where it occurs it is generally in larger numbers than Tabanus.

Lyperosia is found in arid camel country, and is dependent on the rainfall to the extent that it swarms in years when there is a good rainfall, while in poor years it is scanty.

Hippobosca camelina is confined to the Trans-Indus portion of the Punjab. *H. maculata* occasionally bites, but does not live on the camel.

The author thinks that in places where bovines are the only reservoir of infection, or where camels seldom come into contact with horses, chance plays an important part in the occurrence of equine outbreaks. The reason for this is that surra oxen generally carry only a few trypanosomes, and only a small proportion of the flies which bite them are likely to become dangerous as disease spreaders. Camels on the other hand frequently have large numbers of the parasite in their blood, but they do not come into close contact with horses.

Once the first horse has become infected, however, the disease spreads rapidly provided circumstances are favourable, because the first horse has large numbers of trypanosomes in its blood and the animals stand together in such a way as to make mechanical

transmission easy. Clinical observations support this view. Surra does not appear in horses in every fly zone annually; if the first case in the horse occurs early the disease generally runs through the stud, while if it be late the outbreak is limited, there being few flies left to effect the transmission.

The circumstances under which camels live make it easier for mechanical transmission to be effected.

The author suggests the possibility that the *T. evansi* may be in reality only a variety of the *T. brucei* which has acquired its characteristics owing to innumerable passages through camels.

(8) Mesnil (F.) & Leger (M.). **Documents relatifs au Surra des Caprins et à leur Immunité.** [Surra in the Goat, and Immunity.]—*Bull. Soc. Path. Exot.* 1912. Jan. Vol. 5. No. 1, pp. 31–35.

Although there is ample evidence that ruminants frequently recover from trypanosome infections and thereby acquire a strong immunity, there are but scanty references in literature to the duration of such immunity.

Laveran and Mesnil record the case of a goat which having been the subject of nagana for a period of five months was still immune to that disease 21 months later, and was infected in the interval with mal de caderas and surra.

A second goat, as recorded by the same authors, was infected with nagana for a period of four months (Dec.-April) and with surra from Oct.-March. More than a year after its recovery from nagana its serum proved protective for a mouse against the *T. brucei*. The serum of this goat was again tested and found to be protective after 18 months.

According to Wryburg two zebus which had recovered from surra in Sumatra were immune for a period which did not appear to exceed two years.

Mesnil records that two bovines inoculated at Alfort recovered. Their immunity was tested on three occasions by inoculations with *T. evansi*, to which they proved resistant. One of these animals was again tested a month later with the Mauritius strain and again resisted infection. This not only established the identity of the trypanosomes, but showed that the animal had retained its immunity for a period of more than two years.

Laveran records the persistence of immunity in a goat, which had been infected with *T. evansi*, for 2 years and 4 months, and in a sheep which had recovered from a dimorphon infection for 22 months. The serum of this sheep taken 8 months after recovery protected mice in doses of 0·25 cc. He also mentions a goat which recovered from a dimorphon infection and whose serum proved protective 17 months later.

Finally the immunity of a Macacus against the *T. gambiense* may persist for more than a year.

The authors record the following experiments of their own.

Goat S.—Inoculated Nov. 26th 1907 with *T. congolense*; infection lasted about 5 months. Reinoculations practised 8 and 12 months later proved negative.

In Jan. 1909 the animal was inoculated with Indian surra, with the result that it was infected for a period of about 4 months. Mice and a dog inoculated from it before May 1909 were infected, but a dog inoculated in June remained uninfected.

Goat J.—Inoculated with Indian surra at the end of January 1909 became infected and recovered in about 4 months. The results of the inoculation of test animals were the same as in the preceding case.

Goat R.—Inoculated with Taher virus of Algerian horses (Ed. and Et. SERGENT). The animal contracted an infection which lasted for 3 months. Nine months later it was infected with the same virus and again recovered.

Goat B.—Inoculated in May 1909 with a strain derived from Senegal. The infection lasted for more than six months, and a number of animals (mice and a dog) inoculated from it before December died from the disease; mice and a dog inoculated in March 1910 did not react.

Before the goats were reinoculated with Indian surra in May 1911, the protective power of their serum was tested. In the case of two of the animals the serum was found to stay the course of the disease slightly when injected into the peritoneum of mice together with the *T. evansi*. The serum of the other two was without any protective effect.

The inoculation of the goats shewed the absolute concordance between the protective power of their serum and the immunity acquired by them.

Animals S and J, which had recovered from surra about 2 years previously, and the serum of which retarded the disease in the mouse, did not become reinfected, nor did animals inoculated from them. The other two (R and B) became infected. Trypanosomes were found in the blood of goat R, but not in that of goat B.

The duration of the disease in these two goats was determined by the inoculation of experimental animals. In the case of goat R it was found that dogs and mice inoculated in November of the same year did not become infected.

Goat B.—One mouse inoculated on the eighth day after the goat was inoculated remained healthy, but a number of mice and a dog inoculated subsequently contracted the disease. The period of incubation was long and the disease developed slowly; parasites were not constantly present in the peripheral blood. Animals inoculated in November failed to react.

Two of the goats originally inoculated with surra did not react, the other two giving positive results. With regard to goat R, it is known that the trypanosome of Taher is different from that of surra. The result in the case of B was more unexpected, since the trypanosome used for the primary infection has been shewn by one of the authors to belong to the species *evansi*. In any case the result would not invalidate the result obtained in the first case. The goat had not any greater immunity against the Mauritius strain. A difference in the condition of the two animals immediately before inoculation may have had some

influence on the marked difference in the infection in the two cases. The authors point out, however, that the disease in the two goats lasted a similar length of time, and suggest that something may be learnt from it regarding the difficult problem of the identification of viruses.

Three months after the inoculation with Indian surra the serum of the four goats was tested as to its protective powers. As in the preceding cases this was done by inoculating mice intraperitoneally with $\frac{1}{2}$ cc. of serum mixed with $\frac{1}{10}$ cc. of citrated mouse blood containing the *T. evansi* (Indian and Mauritius strains).

All the mice so treated were living at the end of 50 days, the controls being dead in 4-5.

The protective power of the serum of goats S and J was evidently increased by the reinoculation although the animals did not have any reinfection.

If it be true, at any rate for the goat, that the protective power of the serum is an index of immunity, and the authors' experiments would appear to corroborate this view, it may be supposed that as the serum of goats S and J became more strongly protective as a result of the reinoculation, the immunity of the animals also increased. Up to the time of writing the protective power of the serum of these two animals had remained at the same level.

Goats S and J were reinoculated in September 1911, the first with nagana (Uganda strain) and the second with the Zululand strain. In both animals there was a slight, but at the same time distinct reaction. The authors finally state that the serum of these goats proved protective against the homologous nagana, but not against the heterologous.

(9) Leese (A. S.). **Third Series of Experiments on Treatment of Surra in Camels, with some Cures.**—*Jl. Trop. Veter. Science.* 1912. Jan. Vol. 7. No. 1. pp. 1–18.

The author refers to the report of the second series of experiments, which was published in the *Journal of Tropical Veterinary Science*, 1910, Vol. 5, p. 397, and which shewed that neither white arsenic nor yellow orpiment given in bolus had any trypanosome-dispersing effect; but that atoxyl (intravenously), tartar emetic (intravenously) and sodium arsenate (in solution per os) are all useful. The dosage necessary for dispersing trypanosomes with these drugs was ascertained and combined treatments shewed fairly promising results, the return of the trypanosomes into the circulation being delayed as long as from 44-58 days after the last dose.

The present series of experiments was carried out principally with these three drugs.

The atoxyl solutions were always freshly prepared and the sodium arsenate was the amorphous salt.

For convenience of description the author indicates the different methods of treatment by applying to them the number of the first camel cured in each case.

"264" *Method.*—Four grammes to 4·4 of atoxyl intravenously, 0·5 gramme of tartar emetic, and 55 to 60 grains of sodium arsenate in drench were given on three consecutive days at intervals of 2, 4, 8, and 12 days.

Three camels shewed no relapses in from 253-498 days, and one relapsed 19 days after the last dose.

The blood was examined daily, and had been examined for a period daily before treatment was commenced, in order to exclude from the treatment any animal in the process of natural recovery.

Although the author does not attach any great importance to the inoculation of small animals with blood of camels not shewing trypanosomes, when the results of such inoculations are negative, nevertheless a guinea-pig was inoculated from each of the recovered animals with negative result.

One of the camels was inoculated again with surra on the 498th day after the cessation of treatment and again took the disease, shewing that the animal was cured of the first infection by drugs; for had the recovery been a natural one there would have been immunity.

Remarks on the treatment.—The treatment is a reducing one, and almost amounts to keeping the animal in a state of chronic poisoning for a time.

The objection to the treatment is its length, and it cannot be considered as suitable for camels in poor condition.

No fatal case of poisoning occurred, but in one where the treatment was a little different in that 4·4 g. of atoxyl were given in the first two series of doses instead of 4 g. poisoning resulted.

In a second case the first dose of 4 g. of atoxyl failed to disperse the trypanosomes in 24 hours, so the dose was repeated on the following day before the antimony was given. Atoxyl poisoning resulted. It appears to be impracticable to omit the last series of doses with the idea of shortening the treatment.

"643" *Method.*—In this experiment the same drugs were used as in the foregoing, but they were given in rotation at intervals of one day, the last dose of sodium arsenate being given on the 27th day. Only one camel recovered, and it is possible that certain dosage experiments carried out previous to the treatment of this animal may have assisted in its cure.

No case of atoxyl poisoning occurred. The blood of the recovered animal was examined for 535 days without any trypanosomes being found and experimental inoculation was also negative.

With the idea of modifying the "264" method, experiments were made with regard to the dosage of atoxyl administered subcutaneously. It was found that 9 grammes caused slight anorexia for a few days, and that 12-18 g. caused poisoning.

Atoxyl poisoning appears to occur more readily if the drug be given with sodium arsenate than when given alone. It is also caused by the repeated administration of doses that would not cause poisoning if given singly.

"588" *Method.*—The treatment was the same as the "264" method save that 5-6 g. of atoxyl were given subcutaneously. The three drugs were administered three times and intervals of 3 and 7 days were allowed to elapse between each series of administrations.

Five animals were treated. One recovered which had relapsed under the "643" method. One died from disease not referable to the treatment, and three relapsed in 12-69 days.

"436" *Method.*—This resembles the "588" method, but four series of doses were administered and the dose of atoxyl used was 5-7·5 g.

Time has not yet elapsed to allow any statement of the full results, but the method appears to be open to grave objection owing to the likelihood of poisoning.

"957" *Method.*—In this method the sodium arsenate was administered before the atoxyl, with a view to getting the effects of the two arsenic compounds about the same time. The treatment however appears to be too dangerous.

Experiments showed that the dose of tartar emetic could be increased with safety. The author used up to 3 grammes, but found that 1·5 g. was about the limit of safety.

"178" *Method.*—This method closely resembles the previous one and is as follows:—

1st day—55 grains sodium arsenate per os.

2nd day—5 grammes atoxyl subcutaneously.

3rd day—1·5 g. antimony tartrate intravenously,

followed by a repetition of the series after an interval of three days.

It is a good thing to give 2 lbs. of Mag. sulph. 12 hours after the last dose as some camels incline towards atoxyl poisoning.

The first animal treated had no relapse and ten others under the treatment are not yet ready for report.

"203" *Method.*—This is exactly the same as the "643" method save that the dose of antimony is altered to 1·5 grammes. One animal has been treated and has not relapsed (6 months), and there has been a great improvement in condition.

The irritant Effects of Sodium Arsenate.—Of the total number of experiments in which sodium arsenate was given in full doses combined with other drugs 12 per cent. have died owing to its irritant effects, apart from cases of nephritis.

Full doses of sodium arsenate have been given alone in dosage experiments 25 times without fatality. A "full" dose is one that is fairly reliable for the dispersal of trypanosomes.

Experiments are being carried out with smaller doses.

Holmes' "arsenic alone" treatment was found to be impracticable in the camel. White arsenic alone had no effect on the trypanosomes; if given with an equal weight of potassium carbonate it disperses the trypanosomes, but is exceedingly irritant.

(10) Breisinger (K. A.). **Chemotherapeutische Versuche bei experimenteller Trypanosomiasis der Rinder.** [Chemotherapeutic Experiments on Cattle experimentally infected with Trypanosomes.]—*Zeit. f. Hyg. und Infektionskrankh.* 1912. April 25. Vol. 71. No. 3. pp. 367–403.

The experiments were undertaken to ascertain (1) whether certain chemical substances were capable of causing immediate and permanent disappearance of trypanosomes by a single intravenous injection; (2) how the animals withstood doses which achieved this object.

All the animals used were inoculated with nagana, the strain being one that was slightly virulent for cattle, but which killed mice in 3-5 days.

Ox 1 was treated with arsenophenylglycin and tartar emetic. Oxen 2 and 4 were treated with salvarsan, and No. 10 with a new dye named "Trypaflavin B," which together with the salvarsan was obtained from Ehrlich.

All the animals were examined for flagellates both by culture and by experimental inoculation before being used for the experiments, with negative results.

A somewhat extensive review of the literature is given.

Ox 1.—This animal was infected by inoculation with the heart blood of a mouse infected with nagana. Trypanosomes were demonstrable in the peripheral blood on the 5th day after infection.

Treatment was begun on the 8th day, trypanosomes being then visible in the blood.

A dose of 18·55 grammes of arsenophenylglycin was injected intravenously in 600 g. of sterile salt solution, the solution being heated to 38° C. before injection. Three-quarters of an hour later 5·3 g. of tartar emetic were administered in the same way dissolved in 530 g. of distilled water heated to 37° C.

The animal very shortly showed signs of uneasiness and colic, and died 2 hours later.

The arsenophenylglycin was administered in the proportion of 0·035 g. per kilog. body-weight, and the emetic in the proportion of 0·01 g. The principal lesions found were: Oedema of the lungs and numerous blood clots in the small bronchi. In addition to these there were numerous haemorrhages in the various tissues and organs.

From the descriptions given in literature by other authors it would appear that death was caused by the arsenophenylglycin, it being questionable whether the tartar emetic was in any way responsible.

Mice inoculated at the time when the post-mortem was made—four hours after death—remained healthy and free of trypanosomes, and since motile trypanosomes are generally discoverable in the blood for a much longer time after death it would appear that the treatment had the effect of clearing the blood stream of parasites.

Treatment with Salvarsan (Ehrlich). Ox 2.—This animal was inoculated with nagana as in the case of No. 1. As no trypanosomes were visible in the blood by the 10th day a mouse was

inoculated with blood drawn from the ear and the animal was also reinoculated. The inoculation of this mouse showed that as a matter of fact the first inoculation was successful. Trypanosomes were found to be present in small numbers on the 5th day after the second inoculation. Mice were also inoculated after the second inoculation of the ox. The mice inoculated after the first inoculation died on the 12th and 16th days, showing that the trypanosomes were very scanty in the blood. Those done after the second inoculation died in 5-6 days.

On the 32nd day after the first infection parasites were found to be numerous in the blood and treatment was resorted to.

Salvarsan was administered in the proportion in which it is administered to the human subject, *i.e.*, 0·01 g. per kilog. body-weight. The solution used was prepared in the following way:—

4·4 g. of salvarsan were taken from vacuum tubes, in which it is kept protected from the light, and mixed with 400 cc. of 0·9 per cent. sterile salt solution, the mixture being agitated until solution was complete. To this were added 8·27 cc. of standard caustic soda solution. The resulting precipitate was dissolved by the addition of a few drops of caustic soda save for a few flakes which settled to the bottom. To the clear solution 600 cc. of sterile salt solution were then added. The mixture was warmed to 37° C. and then injected slowly into the left jugular vein. The actual quantity of salvarsan introduced was only 4·3 g. since there was a little precipitate.

Three-quarters of an hour after the injection there were severe muscular rigors of the body and limbs, the animal had an anxious expression and there was straining, and the animal went down.

An hour and a half later the animal had become quite quiet again and the rigors had absolutely disappeared.

The temperature rose to 40·2° an hour and a half after the injection and then gradually sank to normal again. The animal appeared to be quite lively 7½ hours after injection and its appetite was good. There was no evidence of local inflammation.

Test of the therapeutic Value of Salvarsan Treatment—

Mice were inoculated 3 and 6 hours after the injection of the drug, films and moist preparations being also examined.

Between the 23rd and the 72nd hours after the injection 9 thick preparations from which the corpuscles were removed by solution, 13 blood films, and 8 hanging-drop preparations were examined.

From the 4th to the 10th day the blood was examined daily, and then fresh blood and thick preparations were examined every 3rd or 4th day. Eight days after the injection of salvarsan broth tubes were inoculated with defibrinated blood and two mice were inoculated.

None of the mice showed trypanosomes in its blood, and all remained healthy for four months.

No trypanosomes could be discovered in the films or moist preparation made 3 and 6 hours after treatment. There were immense numbers of collections of round blue-stained granules in practically every preparation, which the author considers to be remains of the disintegrated bodies of the trypanosomes.

Further examination of blood smears and the inoculation of control mice showed that trypanosomes did not reappear in the blood of the ox during the whole period that it was under observation (16 weeks). Five and a half weeks after the treatment the author discovered in the blood rounded bodies with or without a flagellum, and some with two flagella which he believes to be connected in some way with trypanosomes.

Ox 4.—After the blood of this animal had been examined for trypanosomes or "culture flagellates" with negative results, it was inoculated intravenously with 30 cc. of culture medium containing "culture flagellates" obtained from a calf.

This inoculation was a failure.

The animal was then inoculated with the heart blood of two mice infected with trypanosomiasis.

Trypanosomes were discovered in the blood on the 3rd day. On the 6th day broth cultures were made and on the following day no trypanosomes could be found in the tubes. There was no subsequent development of "culture flagellates" in the tubes.

As the number of trypanosomes in the blood fell off, the ox was reinoculated intraperitoneally with the heart blood of three trypanosome-infected mice.

Trypanosomes were numerously present in the blood on the 12th day after the first infection.

This animal was treated in the same way as No. 2 save that the dose given was half that per kilog. body weight.

There was a rise of temperature and some general disturbance.

Mice were inoculated and blood preparations examined as in the case of Ox 2.

In thick preparations made 2, 3 and 6 hours after treatment there were observed large numbers of well defined rounded trypanosome forms, some of which had no flagellum and others had one or two.

By the 19th hour these bodies were scantily present, but they were found 28 hours after and also on the 2nd and 3rd days. No trypanosomes could be discovered.

No trypanosomes were found in the inoculated mice, nor were rounded forms observed although they were present in the blood used for inoculation.

Numerous trypanosomes were found in the blood of a mouse inoculated from the ox 9 weeks after treatment, and dead on the 9th day. There was therefore a relapse.

Treatment with Trypaflavin B (Ehrlich). Trypaflavin is a light crystalline cinnabar-red powder that is fairly soluble in salt solution.

In hanging-drop preparations containing one drop each of 0·4 per cent. trypaflavin solution in 0·9 per cent. salt solution, prepared an hour previously, and of mouse blood containing large numbers of nagana trypanosomes, the parasite exhibited at the end of ten minutes only sluggish movements and rosette clumps were formed. After 15-20 the trypanosomes were practically motionless and after 30 minutes quite motionless.

Ox 10.—Before infection neither trypanosomes nor culture flagellates could be discovered in the blood.

The animal was infected with the heart blood of a mouse in which trypanosomes were numerous.

From the 4th-7th day after infection rounded flagellate and non-flagellate forms were discovered in thick preparations. From this day on up to the time of treatment trypanosomes were present in the blood. No culture flagellates were found in cultures made on the 8th day after infection, nor were nagana trypanosomes discoverable in the tubes two days later. In order to test whether the milk contained trypanosomes two mice were inoculated with 1·5 cc. of centrifuge deposit but both remained healthy.

The dose of trypaflavin used was 0·01 g. per kilog. body-weight.

A 0·4 per cent. solution was made by simply shaking the powder up with salt solution warmed to 40° C.

The solution was injected into the jugular veins, but owing to the restlessness of the animal a portion escaped into the subcutaneous tissue and a portion was lost. The animal probably received only about 3·5 g. of the trypaflavin.

Immediately after the injection an egg-like swelling about 10 centimetres long formed at the seat of operation.

There was no rise of temperature, but from time to time there were rigors.

On the following day there was on the right side a hard painful cord, principally in the connective tissue but also involving the jugular vein, about 20 centimetres broad and 8-10 thick. At the seat of injection on the left side there was a swelling about the size of a man's fist. The swelling extended until on the 10th day after injection the inferior border of the neck was quite rounded and the trachea surrounded. At its lower end the swelling was sack-like. The head was held low and extended owing to the weight and immobility of the neck.

The animal was handed over to the clinique for treatment, and the application of a cantharides ointment caused a decrease in the size of the swelling by the 4th day. Subsequently there was only a little thickening of the tissues remaining.

Mice inoculated 3 and 9 days after treatment remained healthy. Of the two inoculated 6 hours after treatment one remained healthy and the other became infected and died on the 11th day.

In smear preparations made 2 hours after treatment there were numerous collections of blue granules, which sometimes showed red-stained granules among them, and also the remains of trypanosomes in the form of collections of blue-stained granules arranged in a row and containing an obvious nucleus and centrosome. No intact trypanosomes were found.

In a thick preparation made 31 hours after treatment there were found three solid-looking rounded forms in a clump, having a darkly stained protoplasmic body, a large and a small red-stained nucleus, and a single flagellum. On the second day after treatment a vacuolated body of an oval shape with a large red-stained nucleus was found. Non-flagellated forms were found 20 days after treatment, and uni- and biflagellate forms were found after 4 weeks.

Trypanosomes were subsequently proved to be present in the blood by experimental inoculation of mice.

Culture flagellates were proved to be present in the blood of this animal in the summer of 1910 and also 1911, but they disappeared during the winter.

Infection Experiments with Oxen 3 *and* 6.—The experiments carried out with these two animals deserve special mention on account of the peculiar results obtained.

Ox 3 had been previously infected successfully with nagana three times and Ox 6 once. The animals recovered spontaneously.

Ox 3 was inoculated with nagana from a mouse, but the inoculation failed as shown by inoculation of a mouse. The inoculation was repeated a fortnight later with similar results, but 9 days after the second inoculation the author discovered in thick preparations oval bodies which in many instances were provided with two flagella and which possibly stood in some relationship to trypanosomes.

Thinking that the negative results of the nagana inoculations were due to the low virulence of the strain used, the author again inoculated the animal with blood both subcutaneously and intravenously from Ox 10 which had large numbers of parasites in its blood at the moment.

Six days later round flagellated bodies were found in thick preparations, but neither culture flagellates nor trypanosomes could be demonstrated in the blood either by inoculation or by culture. The animal was finally inoculated intraperitoneally on two occasions with 50 cc. of blood from Ox 4, there being large numbers of trypanosomes in the blood at the time.

Again the animal did not react, but from the 2nd to the 5th and again on from the 10th day flagellated and non-flagellated bodies were found in thick preparations.

Culture trypanosomes had been found in the blood of Ox 6 during the summer of 1910 and this animal was used to test its susceptibility to the trypanosomes found in the blood of German cattle by Behn. It was accordingly inoculated intravenously with 75 cc. of defibrinated filtered blood diluted with salt solution, the blood being taken from Calf 11.

The blood was examined for four weeks, but neither culture trypanosomes nor blood trypanosomes could be demonstrated by any method.

Repeated attempts, similar to those made in the case of Ox 3, to infect Ox 6 with nagana failed, but on the 3rd day after the first attempt and from the 1st-6th days, and again on the 10th day, numerous flagellated round forms were found in thick preparations.

Experiments to infect mice and hedgehogs with the rounded bodies failed, but it is worthy of notice that subsequently culture flagellates were again demonstrable in the blood of Ox 6 by cultivation.

The author believes that the failures to infect these two animals were due to immunity acquired by the previous inoculations.

Experiments showed that the serum of these animals conferred no appreciable immunity on white mice.

The author gives a brief survey of the literature regarding the rounded forms and then gives details of those observed by him. They varied from 6-10 microns in length and 4-6 in breadth. The cytoplasm showed numerous vacuoles and stained pale blue. The nucleus varied greatly in size, the largest being about 4 microns. The protoplasm contained in many instances numerous small chromatin granules, which were frequently surrounded by a clear zone. The blepharoplast was recognisable in many cases only by its dark carmine tint.

In the forms showing two flagella both of these came off from one side of the body, and very frequently one of them occupied a position around the edge of the body. This was also observed in the uniflagellate bodies. Specimens were observed with four flagella. The bodies were observed in the blood both in cases in which treatment had been resorted to and in cases in which no treatment had been applied.

The author is of the opinion that the rounded forms are fully-grown or dividing trypanosomes which owing to unsuitable conditions have undergone regressive morphological and functional changes.

It is worthy of note that the rounded forms were never observed by the author in thin blood smears, but only in thick preparations.

Conclusions.

I. Chemo-therapeutic treatment of experimental trypanosomiasis in cattle infected with the "Ferox" strain of nagana.—

1. Arsenophenylglycin in combination with tartar emetic is dangerous owing to the large dose required to ensure a cure. The lesions found in animals dying as a result of the treatment are oedema of the lungs, blood clots in the small bronchi, and haemorrhages in the organs and tissues.

2. Salvarsan caused the immediate disappearance of the parasites from the blood stream in Oxen 2 and 4. There was no evidence of intoxication or local reaction in these animals. In Ox 2 there was a very rapid rise of temperature which sank within 2 days to normal.

Nagana trypanosomes were not found subsequently in the blood of Ox 2, which received salvarsan in a dose of 0·01 g. per kilog. body-weight. There was a relapse in the case of Ox 4, which received only half this dose of the drug.

3. Trypaflavin B is of little value owing to the severe local inflammation caused by the injection.

II. Inoculation of Ox 4 with culture flagellates failed entirely, neither blood trypanosomes nor culture flagellates making their appearance.

III. Inoculation of Ox 6 with blood of a calf in which neither blood trypanosomes nor culture flagellates could be demonstrated, but which had been proved infective by inoculation, failed.

IV. Oxen 3 and 6, which had spontaneously recovered from infection with nagana (Ferox strain), failed to react to the same strain, when inoculated with mouse or ox blood, and their immunity was thereby increased.

V. The rounded bodies found in thick preparations of the blood of all the animals, treated and untreated, should be considered as either adult forms or dividing trypanosomes which have undergone regressive changes of morphology and function owing to unsuitable conditions. Trypanosomes could not be demonstrated either in mice or hedgehogs inoculated with ox blood containing these parasites or in broth cultures.

(11) WALKER (E. L.). **The Schizogony of *Trypanosoma evansi* in the Spleen of the Vertebrate Host.**—*Philippine Jl. of Science.* Sec. B. [*Philippine Jl. of Trop. Med.*] 1912. Feb. Vol. 7. No. 1. pp. 53–62.

Mention is first made of the descriptions given by SALVIN-MOORE and BREINL (1907) of developmental stages of the *T. gambiense* in the lungs of infected rats. According to these authors peculiar bodies are formed at or near the maximum multiplication of the parasites in the circulating blood. They describe in the process of their formation the development of a stainable band connecting the nucleus with the centrosome, which subsequently disappears. This is followed by the extrusion of the nucleus, the rest of the trypanosome disintegrating. The oval bodies formed by the nucleus and a small amount of protoplasm are stored in the spleen and bone marrow and are termed "latent bodies." These latent bodies are believed by the authors to be specially resistant forms of the parasite which give rise to new generations. The work of CHAGAS (1909) is next referred to. This author published an account of a new trypanosome of man in Brazil which is transmitted by a biting bug (*Conorhinus megistus*). This parasite is named the *Schizotrypanum cruzi*.

It is said by the author that this trypanosome does not multiply by simple division in the peripheral blood, but undergoes a process of schizogony in the capillaries of the lung.

This reproductive process takes place at the times when the parasite becomes numerous in the blood. The trypanosome sheds its undulating membrane and flagellum, bends upon itself, and becomes fused into a rounded or oval body. In some of the parasites the centrosome is shed with the flagellum, and in others it is retained in the body. By division of the nucleus of the first form and of the nucleus and centrosome of the second, and by differentiation of the protoplasm, there are developed from these bodies schizocysts containing 8 small club-shaped merozoites. These escape and, penetrating red blood corpuscles, develop into trypanosomes which make their escape from the corpuscles when mature. The merozoites which have no centrosome develop into female parasites, having a small centrosome derived from the nucleus, and a broad body containing a round nucleus. Those possessing a centrosome develop into male trypanosomes having a large centrosome, an elongated nucleus, and a slender body.

In 1910 HARTMANN found in a section of lung of a guinea-pig infected with this parasite greatly hypertrophied endothelial cells containing large numbers of more or less pyriform bodies with two nuclei. According to Hartmann, similar intracellular stages were subsequently found by Chagas in the heart muscle and brain of a man dead of the disease. He believes that the intracellular stage represents real schizogony, while the extracellular stage with sexual differentiation is to be considered a gametogony.

CARINI (1911) has described a similar schizogony of *T. leptodactyli* in endothelial cells from the heart blood of an infected animal.

FANTHAM (1911), working with *T. gambiense* and *T. rhodesiense*, has confirmed the observations of Salvin-Moore and Breinl regarding the presence of the rounded bodies in the lungs, but disagrees as to their method of development. According to Fantham, from observations made on circulating blood under the microscope, the anterior end of the trypanosome disintegrates, the centrosome migrates to near the nucleus, and the posterior end of the trypanosome with the remnant of the flagellum is cast off, the remainder forming the latent body. He was also able to observe the change of the latent bodies into trypanosomes by mixing infected spleen pulp with fresh blood of an uninfected rat, and observing it under the microscope. He claims also to have transmitted the disease by the inoculation of spleen pulp containing latent bodies, but no motile trypanosomes.

LAVERAN (1911) concludes that the rounded bodies are involution forms.

BUCHANAN (1911) has observed similar bodies to those already described in the organs of the gerbil infected with the *T. brucei (pecaudi)*, but his explanation of their development is different. The trypanosome bends upon itself and becomes fused, the flagellum remaining attached for a time. Buchanan found in smears from the spleen all stages from the ring-formed parasites to the fairly mature trypanosomes in the red corpuscles.

In the development of the *T. evansi* in the guinea-pig there occur round non-flagellated forms in the spleen and marrow which correspond to the latent bodies described by the other authors, but they are developmental rather than latent, in that they undergo a process of schizogony comparable to that occurring in the *Schizotrypanum cruzi*.

The author has studied two strains of the trypanosomes, one from a horse and the other from a carabao. The strains have been carried on in guinea-pigs, and the development described is based on the examination of the blood and organs of that animal.

The blood taken from the ear veins, and the internal organs of guinea-pigs killed at different periods of infection, have been studied fresh, in dried smears, in smears fixed wet, and in paraffin sections. These preparations have been stained with Giemsa's stain, aqueous alum haematoxylin, Mallory's ferric chloride haematoxylin, and Seidelin's iron haematoxylin. Air-dried smears of both the blood and organs, stained for 12-24 hours

with Giemsa's stain have given the best results. Sections have been useful in determining the relation of the stages of the trypanosome to the tissues.

T. evansi, when inoculated subcutaneously into guinea-pigs, has an incubation period of 5-16 days. The disease runs a more or less chronic course of one to several months duration. The course of the disease is marked by alternating phases of increase and decrease of parasites in the peripheral blood.

It has been found that large numbers of rounded binucleate bodies appear in certain organs at or near the maximum increase of the parasites in the blood. Few or no round bodies could be found during the periods when the parasites were scanty in, or absent from the blood.

The situation of development of these round forms of the *T. evansi* does not correspond with that of the latent bodies of *T. gambiense* and *T. rhodesiense*, nor with that of the schizonts of the *Schizotrypanum cruzi*. The latter are described as occurring in the capillaries of the lungs, but in the case of the *T. evansi* the round forms develop principally in the spleen, and to a less extent in the bone marrow. Sections show that they are developed extracellularly in the small capillaries.

The author's view with regard to the formation of the rounded bodies differs from those expressed by the other authors mentioned. The parasite bends round until its ends meet and then fuses into a rounded body, or into a ring which subsequently becomes solid. The flagellum remains attached around one side of the parasite for a time, but disappears very soon.

The bodies measure 2-5 microns in diameter and have a body, which stains pale blue with Giemsa, containing a central or eccentrically placed nucleus and a centrosome which is often on the opposite side of the body to the nucleus. The nucleus and centrosome stain red with Giemsa. The author has not been able to distinguish the type without a centrosome, as described by Chagas.

In the spleen of guinea-pigs killed when the blood is swarming with trypanosomes a further development of these bodies is evident. The round binucleated body increases in size, and both nucleus and centrosome undergo a process of division. The fully developed schizonts measure 10-15 microns in diameter. The number of nuclei and centrosomes varied from 4-16. Some of the large parasites show evidence of fission of the protoplasm, and others complete differentiation of merozoites, which appear to be surrounded by a thin cyst wall.

The merozoites are arranged like the segments of an orange with a slight spiral twist. They are elongated, sickle-shaped bodies, 6-10 microns long and 1-1·5 wide. They have a central nucleus and a centrosome near one end, but no flagellum or undulating membrane. Forms corresponding to the intracorpuscular stages of Chagas and Buchanan are observed, but the author has not been able to convince himself that the parasites are really within the corpuscles.

In the author's view the merozoites develop directly into trypanosomes.

No sexual processes have been observed preceding or during the formation of the round bodies, nor during the development of the multi-nuclear cysts. Sexual reproduction should, according to the accepted theory, take place in the body of the invertebrate host. The merozoites show no dimorphism, nor has the author observed any sexual differentiation in the mature trypanosomes; he therefore designates the reproductive process a schizogony.

The author is of the opinion that the genus Schizotrypanum may have to be abandoned. The schizogony described by Hartmann and Carini is probably phagocytosis of young schizonts by endothelial macrophagi. The absence of simple division in the peripheral blood would appear to be distinctive of the *S. cruzi;* but in view of the fact that this parasite appears to multiply by simple division in the gut of the invertebrate host and in cultures on Novy and MacNeal's medium, a suspicion is raised as to the accuracy of the observation.

Possibly some of the young schizonts persist through the latent period and undergo schizogony at the beginning of the relapse.

Conclusions.

"In the developmental cycle of *Trypanosoma evansi* a schizogony takes place in the spleen of the vertebrate host.

"The observations of Salvin-Moore and Breinl, Fantham, and Buchanan that forms similar to the young schizonts of *Trypanosoma evansi* occur in the internal organs of animals infected with *Trypanosoma gambiense, T. rhodesiense,* and *T. brucei* make it probable that schizogony is a reproductive process common to the trypanosomata.

"The validity of *Schizotrypanum* Chagas as a genus distinct from *Trypanosoma* Gruby appears to be doubtful.

"Further investigation is necessary to determine the significance of this schizogony in the life-cycle of the trypanosomata and its relation, if any, to latency in trypanosomiasis and to relapses after chemotherapeutic treatment."

(12) Mason (F. Eugene). **Equine Trypanosomiasis in Egypt.**—*Jl. Comp. Path. and Therap.* 1912. June. Vol. 25. No. 2. pp. 93–109.

Having heard it said by the Bedouins that horses after being bitten by flies sometimes developed a disease which presented symptoms identical with those observed in camels affected with el debab, and which was invariably fatal within a few months, the author kept a watch for such cases. The first case encountered was in an Arab horse, the property of the Khedivial Agricultural Society, which was admitted into the Cairo Infirmary. Trypanosomes were easily discoverable in the peripheral blood, and there was also agglutination of the red corpuscles, described by Gaiger as occurring in the blood of horses suffering from surra.

The animal, which appeared to be in good health at the time of purchase, began to lose flesh and to show an irregular temperature some fifteen days later. It was stated that repeated blood examinations had been negative, and that it had been treated with quinine for piroplasmosis. At the time of admission to the Infirmary the animal was very emaciated and weak, the respiration was rapid, the conjunctiva yellow and ecchymosed

and there was oedema of the chest and limbs. Trypanosomes were found in the blood on the following day.

Treatment was commenced on the next day. Two doses of atoxyl followed by orpiment and sodium arsenate were given, the drugs being used in rotation on alternate days, leaving one day's interval. The temperature fell to normal with the first dose of atoxyl. Treatment was continued for about three weeks, when symptoms of arsenic poisoning were observed. In view of the fact that on analysis the orpiment that was used was found to be a mixture of the red and yellow varieties, treatment was discontinued till fresh samples of true yellow orpiment could be obtained from Europe. The animal improved for about five weeks and showed no rise of temperature. When the temperature rose trypanosomes could not be detected in the blood, but there was marked agglutination of the red corpuscles.

The treatment was recommenced five weeks later and was continued for a month. Arsacetin, orpiment, and sodium arsenate were used in rotation at intervals of one day. The dose of arsacetin was from 2·5-3·0 grammes, that of orpiment 6·0-7·0 grammes save in the case of the last two administrations when the doses were 9·0 and 10·0 grammes, the latter being given in two doses; the dose of sodium arsenate was from 2·0-4·0 grammes. After this treatment the animal was in excellent condition, and was discharged for duty.

At the time of writing (6 weeks later) the animal was still apparently perfectly well.

It is of interest to note the following points: It is not certain that the animal was infected in Egypt, as it had been recently imported from Syria, and probably came over with a number of camels.

Before treatment was commenced and at a time when the parasites were not numerous, citrated blood from the jugular was used for the inoculation of a number of animals.

Four white rats were inoculated subcutaneously with citrate mixture corresponding to 1 cc. of blood. These rats died with their blood swarming with trypanosomes in from 22-38 days.

On the same day an English gelding was inoculated subcutaneously with 10 cc. of the same mixture (equal parts of blood and citrate).

On the twelfth day there was an elevation of temperature, but the blood was not examined.

The following morning there was a further rise of temperature and there was marked agglutination of the red cells, but no trypanosomes were found.

On the fourteenth day trypanosomes appeared in the blood.

After this the disease advanced rapidly and clinical symptoms appeared, the animal dying on the twenty-seventh day.

At the post-mortem the following lesions were found: general emaciation, gastro-enteritis, enlargement of the spleen which showed numerous haemorrhagic spots, liver enlarged and stiff, lungs oedematous, lymphatic glands oedematous, heart hypertrophied on the left side and dilated on the right, haemorrhages under the endocardium.

An Egyptian mare was inoculated from the above English gelding on the twenty-fifth day of illness when trypanosomes were numerous. Trypanosomes appeared on the eleventh day.

On the forty-first day she was given 8 grammes of the new sample of orpiment to test its toxicity, and a few days later 15 grammes. There was no colic or diarrhoea. Death occurred on the 60th day.

The lesions were much the same as in the preceding case.

A second mare inoculated from the above Egyptian mare died on the thirtieth day, trypanosomes having appeared on the ninth day. Another mare inoculated with the same material died on the thirty-first day, trypanosomes having been observed on the fifth day.

White rats inoculated from the first Egyptian mare died on the fourteenth, nineteenth, and twenty-first days respectively.

Other naturally occurring Cases.—

Case II. Chronic case. Animal had been more or less ill for about five months when trypanosomes were found.

A dose of 3 grammes of salvarsan administered intravenously caused some improvement, but there was a relapse. Two months later 4·2 grammes were administered with great improvement in condition as a result. Temperature had remained normal up to date (2 months).

Case III. Arab horse fifteen years old. Trypanosomes found about a fortnight after admission to the Infirmary. Treatment begun four weeks later. The treatment adopted was the same as in the first natural case—arsacetin, sodium arsenate, and orpiment. Animal greatly improved in condition at the time of writing.

Case IV. No trypanosomes discovered, but marked agglutination of the red corpuscles. After a fortnight's illness the animal was given a little less than 3 grammes of salvarsan. The full dose was prepared, but the injection had to be discontinued owing to the appearance of urgent respiratory symptoms.

There was a marked temperature reaction on the evening of the same day and also on the sixth day after, the latter lasting till the tenth day.

On the twelfth day 100 cc. of 1 per cent, trypanblue were given subcutaneously and the animal shortly afterwards returned to work and has been working and in excellent condition ever since.

Case V. Animal had been ill (fever) on three occasions before admission to the Infirmary. Trypanosomes found a month after admission. Treatment as in Case I.—Recovery.

Case VI. English thoroughbred. Trypanosomes found. Case said to be doing well under treatment.

The fly seasons in Egypt occur in May and June and again in September.

During these months the *Tabanus taeniola* and *Tabanus ditaeniatus* abound in certain known localities. There appears to be no doubt that they are responsible for the transmission of the disease from camels to horses, and also that the infection of

horses takes place occasionally only. No other domestic species is known to harbour the trypanosome.

The Trypanosome.—

In Case I., the origin of which was doubtful, the shortest parasite observed measured 17·38 μ and the longest 23·8 μ. The width at the widest part was about 1·42 μ. Compared with the measurements of trypanosomes naturally occurring in camels those found in Case I. were both a little shorter and a little more slender. Otherwise there was a very close resemblance. In both cases the body posteriorly to the nucleus was free from granules, and in some specimens the nucleus was prolonged anteriorly by a horn-shaped mass of granules. The undulating membrane was well developed. The cytoplasm extended to the anterior end of the flagellum.

In comparison with the measurements made of trypanosomes from cases of undoubted Egyptian origin those found in Case I. were slightly smaller, but the author suggests that this may have been due to the preparation from which the measurements were made having dried rather more slowly and thus caused more contraction. The fact that in a drawing made under the same magnification with the camera lucida the blood corpuscles appear smaller supports this view.

In preparations made from infected white rats the parasites appeared to be distinctly larger than in those made from the blood with which the rats were inoculated.

It also appeared that passage through a series of horses was responsible for an increase in the length of the parasite, but not in the width.

(13) Watson (A.). **Dourine—Its Pathogenicity, and a Practical Test of the Efficacy of Drug Treatment, with especial reference to the Action of Atoxyl and Arsenophenylglycin.**—*Report of Veter. Director General and Live Stock Commissioner J. G. Rutherford, C.M.G., for Year ending March* 31, 1911. Canada: Dept. of Agriculture. Sessional Paper No. 15c—1912. pp. 151–156.

The paper presents a summary of observations on the pathogenicity of a strain of dourine in horses, and the results of a series of infections with the same strain in which experimental treatment was applied.

Pathogenicity.—The author has endeavoured to follow a plan which he believes to be the proper procedure in the study of any animal trypanosomiasis. He is of the opinion that the disease and its causal trypanosome should be investigated in the following manner. In the first place cases of natural infection should be examined, this being followed by an investigation of the disease set up experimentally in the natural host, and then the experimental infection of unnatural hosts; the entire process being finally reversed. The author is further of the opinion that experimental infection of unnatural hosts is carried to an extreme, and that too high a value is placed upon pure laboratory experimentation.

Throughout his experiments a single strain of the trypanosome was used. The strain was carried on directly from horse to horse, without any intermediary passage through laboratory animals, and was not subjected to any environment other than its natural host.

The strain used was originally isolated from a stallion that had suffered from the chronic form of the disease for about two years, showed all the usual symptoms, and finally died of the disease.

First generation.—Twelve mares naturally infected. Diagnosis was based on clinical symptoms.

Six of the mares were killed when the disease, which was chronic and intermittent in all cases, had been in existence for about a year. Three of the animals died of dourine and three recovered. In the three animals which recovered the disease lasted from twelve to twenty-one months, while the period of survival and recovery was from two to three and a half years. In the fatal cases the disease lasted from two to three and a half years. The trypanosome was first discovered in an animal which survived for three and a half years, the discovery being made in the seventh and eighth months. The trypanosome which was then found was the starting point of the succeeding generations.

Second generation.—A stallion, naturally infected by one of the above-mentioned mares. The animal was under observation for a short time only before its death, but there was a history of the disease having been in existence for about two years.

Four experimental infections.—Two foals, one mare, and one gelding.

In the first foal the course of the disease was fairly rapid, the animal showing marked elevation of temperature, oedema, enlarged glands, paralysis, emaciation; and death took place in the fifth month.

In the second foal and the mare the disease took the following course. First stage—periodicity of trypanosomes in the vaginal fluids and vulvar oedema, local symptoms only. This was followed by a long latent period, which was succeeded by a period of plaque formation, and typical ocular and nervous symptoms. Towards the end of this period trypanosomes were found in each animal in the contents of the local swellings in large agglomerations, and in different stages of phagocytosis. This throws some light on the immunity acquired by these animals.

Recovery was rapid, and at the time of writing had been maintained for two years.

The gelding shewed enlarged glands after three months, and intermittent inco-ordination and paralytic symptoms from the sixth to the twelfth month. Recovery ensued, maintained two and three-quarter years.

Third generation.—Four experimental infections—two fillies and two foals.

In the fillies the disease lasted six to eight months, and there was no evidence of nervous derangement nor disturbance of general health. Recovery.

In the foals the disease was of the severe, intermittent, chronic type. Foal 1—Fourteen paroxysms of fever each lasting about five days, with intermissions of twenty-one days during the first year. Nine paroxysms of six days each at intervals of thirty days during the second year. Remission of symptoms and general improvement of health during the third year; rare brief relapses. Foal 2—Slight elevation of temperature for long periods, and four paroxysms during the first year. Seven, at intervals of about forty-four days and lasting eight days, during the second year. Both foals shewed the usual typical symptoms.

Fourth generation.—One experimental infection of a foal, the course of the disease being similar to the preceding. Recovery indicated in the third year.

Fifth generation.—One experimental infection of a foal. Disease severe throughout. Death in the eighteenth month.

Sixth generation.—One experimental infection of a foal. Disease severe at the commencement after a short period of incubation. Subsequently remissions and intermissions and a period of recovery. Trypanosomes not seen after the third month. Recovery probable.

Seventh generation.—One foal. Disease severe and acute. Death on the forty-first day.

Eighth generation and *ninth generation* almost exactly reproduced the seventh.

Tenth generation.—Very like preceding, but death delayed till the 101st day.

The above observations are in agreement with the general experience that the disease is more progressive in the stallion than the mare.

The virulence of the trypanosome was increased by the passages.

Throughout the series the most striking feature was the development and intensification of a characteristic type of fever. Until the third generation was reached fever was a rare symptom; in the third and fourth it was associated with alternating paroxysms and intermissions; in the fifth and sixth generations it was still more constant; and in the last generations it was the outstanding feature. The trypanosome was more easily found in the later cases than in the early ones, but it was never found in the general circulation. Save on two occasions, when they were found in clear lymph escaping from punctured lymph vessels in the tail, they were observed only in the fluid contents of oedematous swellings and in preparations from vaginal mucosa.

There was no intensification of virulence for laboratory animals, and it was found to be impossible to carry on the strain satisfactorily in them.

The mortality was about 50 per cent. or rather less.

Treatment.—Horses only were used for experiments in the treatment of the disease, for the reasons that the strain was difficult to keep running in laboratory animals and the drugs employed had been proved to be more or less efficient for the treatment of other trypanosomiases.

Atoxyl.—1. The drug in comparatively small doses could not be tolerated by a stallion in the very advanced stages of the disease, and was probably contributory to the immediate cause of death.

2. A filly which received three injections during the sixth month of the disease rapidly recovered. Two foals were obtained from this animal without either the offspring or the stallion becoming infected.

3. A gelding shewing marked paralysis received 10 grammes in seven doses. There was temporary improvement only.

4. A young filly in which the course of the disease was marked by regularly recurring paroxysms with intervals of twenty days received two full doses of atoxyl on the tenth and thirteenth day respectively after an intermission. The next paroxysm was not delayed in the least. Parasites, which had been frequently observed up to the time of administration of the drugs, were not again seen for more than three months.

Atoxyl, Mercury Bichloride, and Potassium Iodide.—5. A mare shewing paralysis to a marked degree received two courses of atoxyl, 18 grammes in all, with injections of bichloride of mercury and potassium iodide in the intervals. There was a complete recovery, which at the time of writing had lasted for two and a half years.

Atoxyl, Mercury and Arsenic Iodide, and Arsenophenylglycin.—6. In a young animal a course of atoxyl given during the fourth month of the disease was without effect. The second course of injections, alternating with injections of a solution containing one per cent. arsenic iodide and mercury iodide, during the sixth and seventh months was also without effect; as was also a third course of large doses of atoxyl during the ninth month. A single large dose of arsenophenylglycin was given at the twelfth month and repeated seven weeks later. The disease made no further progress and the relapses became more infrequent.

Arsenophenylglycin, Trypanblue, and Sodium Arsenate.—7. A filly eleven months old and in the fifth month of the disease received 10 grammes of arsenophenylglycin, and, after an interval of thirty-five days, 12 grammes. About a month later there was a febrile period lasting three days. Arsenious acid and iron sulphate administered daily for a month caused a slight improvement. A mixture of arsenophenylglycin 10 grammes, trypanblue 5 grammes, and sodium arsenate 0·5 gramme was then given. There was a period of remission followed by relapses and death.

Arsenophenylglycin.—8. A filly with severe symptoms and persistent fever received 12 grammes of the drug on the eighty-third day, and forty days later a second dose of 15 grammes. The temperature curve was normal after the first dose, but fever returned on the thirty-third day after the second. On the fourth day of the paroxysm 10 grammes were given. Slight elevation after twelve days, followed by intermission of two months. General oedematous urticaria and fever. Without further treatment the animal apparently recovered.

9. A foal developing acute infection received 7 grammes. The symptoms progressed and a second injection of 9 grammes was given ten days later. Death after two days.

Poisoning with Arsenophenylglycin.—10. Experiment to ascertain the largest dose that could be given with safety.

A healthy stallion received a dose of 30 grammes—80 milligrammes per kilogramme body-weight. No immediate effects. Dullness and depression the following day. On the third day spasmodic contraction of the pharyngeal muscles and difficulty in swallowing, associated with thickening of the lips. In the evening of the same day excitement, rigors, nervous symptoms and death.

Dosage and Administration.—The author found that the administration of 70-75 milligrammes per kilogramme was tolerated, but with some risk, and that 60-65 milligrammes were well borne. He also found that the maximum dose that can be given to a lightweight animal may be toxic if given in the same proportion to a large heavy animal. That is to say, as the body weight increases the proportionate dose should be slightly decreased.

In all cases the drug was given intravenously with a syphon, a one per cent. solution being used.

Remarks.—

"The few experiments cited above do not afford evidence, from a practical standpoint, of a satisfactory method of treatment; some cases have recovered under drug influence, others without any treatment being given at all, and there are cases in which the progress of the disease has scarcely abated, or been but slightly modified, by any of the drugs employed except arsenophenylglycin. A single dose of this preparation will cause the rapid disappearance of trypanosomes from the tissues haunted by them for weeks or months previously, oedema and local symptoms will disappear in unison, yet the characteristic type of fever recurs at stated intervals, and though the parasites are never to be seen again the disease may progress to a fatal termination.

"The variations in individual resistance and in different degrees of virulence are no doubt responsible for much of the apparent contradiction in results. It may be mentioned in passing that in some parts of Africa the treatment of sleeping-sickness with arsenophenylglycin has been attended with marked success, while in others it has as signally failed.

"Dourine, while not by any means the most fatal of the animal trypanosomiases, is one of the most chronic, and apparently the most resistant of any of them to the influence of drug-treatment."

(14) Duke (H. L.). **The Transmission of *Trypanosoma nanum* Laveran.**—*Proc. Roy. Soc.* 1912. April 10. Ser. B. Vol. 85. No. B. 576. pp. 4-9.

The trypanosome was obtained from van Someren, who had found it to be the cause of a fatal disease in the neighbourhood of the Sebwe River. It was found to be identical with *T. nanum* both in its morphology and pathogenicity.

Morphology.—Corresponds very closely to *T. pecorum*.

There is no free flagellum visible. Average length about 14 microns.

Pathogenicity.—The original animal received from van Someren—a goat—was in perfect health at the time of writing—7 months

after infection. From this goat 3 monkeys, 3 white rats, 2 puppies, 1 wild pig, 1 sheep, and 1 goat were inoculated. Of these only the sheep and the goat shewed trypanosomes. The sheep was still in good health 154 days after inoculation, the period of incubation having been 10 days. The goat shewed trypanosomes in its blood after 6 days and died on the 64th day as a result of the infection.

Transmission by G. palpalis.—The first six attempts at transmission failed. Whereas in the first experiment the flies were first fed on a goat, in the second they were fed on the sheep mentioned above.

It was found that the flies fed far more readily on the sheep.

Four experiments were made and the flies were placed on sheep, calves, goats, and dogs. One calf which was used in all four of the experiments became infected.

As a result of the dissection of flies used it would appear that the development of the *T. nanum* in the *G. palpalis* commences in the hind gut and extends forward via the thoracic gut and proventriculus until the proboscis is reached. The salivary glands are apparently not invaded by the trypanosome. A fly with a negative proboscis is presumably non-infective, this conclusion being supported by the fact that on three occasions injection of positive proventriculi failed to infect a goat.

It was found that flagellates may be well established in the proboscis by the 25th day after feeding. Results also indicated that the infection of the proventriculus may be merely a temporary invasion, while the flagellates are becoming established in the proboscis.

(15) Fraser (A. D.) & Duke (H. L.). **An Antelope Trypanosome.**—*Reports of the Sleeping Sickness Commission of the Royal Society.* 1912. No. 12. pp. 56–63. With 10 plates. Abstract in *Proc. Roy. Soc.* 1912. April 10. Ser. B. Vol. 85. No. B. 576. pp. 1–2.

A goat inoculated with blood obtained from a bushbuck shot on the shore of Victoria Nyanza showed trypanosomes in its blood ten days later. The trypanosome corresponded morphologically with the *T. uniforme*.

Cattle, goats, sheep, and bushbuck were infected. Monkeys, pigs, dogs, cats, guinea-pigs, and white rats proved refractory. It was shown experimentally that the *G. palpalis* was capable of transmitting the parasite. The flies became infected in 27-37 days, and the infection in the fly was limited to the proboscis. Flies caught in the same district were found to be naturally infected and capable of setting up infection in a goat. *Trypanosoma vivax* appeared in the goat's blood a few days later.

Conclusions.

1. This trypanosome, which is of fairly frequent occurrence among Lake-shore antelope, is *Trypanosoma uniforme*.
2. The available evidence points to *Glossina palpalis* as being the carrier of this trypanosome.
3. *Glossina palpalis* caught on the Lake-shore are naturally infected with *Trypanosoma uniforme*.

(16) Rodhain (J.), Pons (C.), van den Branden (F.) & Bequaert (J.). **Les Trypanoses animales au Bas-Katanga et leurs Rapports avec les Glossines.** [Animal Trypanosomes at Bas-Katanga and the Part played by Glossina in their Transmission.]—*Bull. Soc. Path. Exot.* 1912. Jan. Vol. 5. No. 1. pp. 45-50.

Two types of trypanosomes were found: one of the *congolense* and the other of the *cazalboui* type.

1. *Trypanosomes of the* Congolense *Type.*—Trypanosomes of this type were found in dogs and goats. The disease produced may be acute or chronic. In the dog the acute cases observed terminated fatally within a month, the chronic cases showing the usual symptoms of trypanosomiasis and slight posterior paralysis.

In preparations fixed in osmic acid the average length of the parasite was about 12·5 microns, the width at the level of the nucleus being 1·5.

The trypanosome was pathogenic for the guinea-pig, death taking place in about three weeks.

The majority of cases observed in the goat were chronic. The trypanosome was about the same size. Three guinea-pigs inoculated from two different goats failed to react, as did a young indigenous dog.

Glossina palpalis and *G. morsitans* were both present at the places where two of the diseased dogs and one of the goats were found. The other animals that were found infected had been transferred several months previously to places where the *G. morsitans* was present exclusively. Tabanus was present in very scanty numbers, while the tsetses were abundantly present.

The authors were able to obtain evidence that the *G. morsitans* is capable of transmitting trypanosomes of the congolense group. Flies were fed on animals affected with trypanosomes of both types and when afterwards examined were found to have immense numbers of trypanosomes in the anterior portion of the mid-gut, and the parasites were swarming in the proboscis.

The authors made no inoculations with material obtained from the infected proboscides, but they hope to furnish laboratory proof that the infection of the flies is a double one. The proportion of flies found to be infected was high—41 per cent.

Treatment Experiments.—Subcutaneous injection of a 5 gm. dose of arsenophenylglycin caused a disappearance of the trypanosomes, but they reappeared in the circulation 15 days later and the goat died in 36 days. Two goats received respectively one and two doses of emetic intravenously, but relapses occurred in both cases. These animals were subsequently treated with tryparosan administered by the mouth.

A complete recovery followed the administration of 8 and 6 g. of the drug, the administration being spread over two days. Two other animals recovered which were treated with emetic followed by tryparosan, but the authors attribute the cures to the dye.

Experiment showed that doses of the dye not exceeding 0·5 g. per kilog. were well tolerated, and it was further found that 0·008 g. of emetic per kilog. could be administered for several

consecutive days without bad results. On the other hand the administration of 0·10 g. of emetic to a dog intravenously was followed by serious disturbance.

2. *Trypanosomes of the* Cazalboui *Type.*—This type of trypanosome, which is easily recognisable in moist preparations by its rapid translatory movement, was found by the authors in the blood of a number of different species of antelope, an eland, and goats.

The parasites were present in small numbers only save in the case of one animal. Inoculation of goats was positive, but subcutaneous inoculation of guinea-pigs failed. Neither the animals in which the parasites were discovered, nor goats infected from them appeared to suffer as a result of the infection.

The largest trypanosome observed in the blood of an antelope measured 31 microns by 2·1, but the average size was 24·4.

No *G. palpalis* were found near Lake Kabwe, but *G. morsitans* was abundantly present. The observations were made during the dry season. Pangonia had disappeared, and Tabanidae were very rarely found. It is probable that the *G. morsitans* is the transmitting agent. Flies which fed upon a goat infected with the *T. cazalboui*, when afterwards placed on a goat which had recovered from the *T. cazalboui* under treatment with emetic and tryparosan, infected it.

Experiments with the same flies on guinea-pigs failed.

Five of the flies were examined after death and infection of the proboscis only was found in three of them. Subcutaneous inoculation of a goat with a proboscis determined infection after 13 days incubation. Both tryparosan and emetic have very rapid effect on the trypanosome, causing complete disappearance.

The authors note that a recovery determined by drug treatment does not set up immunity.

(17) Laveran (A.). **Expériences d'Immunité croisée avec *Trypanosoma brucei*, *Tr. brucei* var. *Werbitzkii*, et *Tr. rhodesiense*.** [Cross-Immunity Experiments with *T. brucei*, *T. brucei* var. *werbitzkii*, and *T. rhodesiense*.]—*Bull. Soc. Path. Exot.* 1912. Feb. Vol. 5. No. 2. pp. 101–105.

Two sheep were inoculated with *T. brucei* and *T. brucei* var. *werbitzkii* respectively. After their recovery they were found to be immune to the trypanosome with which they had been infected. They were then inoculated each with the other trypanosome and it was found that the animal primarily inoculated with the *T. brucei* did not become infected with the *T. brucei* var. *werbitzkii*, and that the one inoculated with the latter became infected with the *T. brucei*, but that the infection was a slight one.

It was also found that the serum of the sheep inoculated with nagana (*Werbitzki*) was active when mixed with the acentrosomic *T. brucei* and feebly active with the *T. brucei*. On the other hand the serum of the sheep inoculated with nagana was found to be active in mixture with the acentrosomic trypanosomes and less active with the nagana parasite.

Low's suggestion that the *T. rhodesiense* was simply the *T. brucei* was tested by inoculating both the sheep with *T. rhodesiense*. Both the animals became infected and died.

(18) Darling (S. T.). **The Pathological Anatomy of Natural and Experimental Murrina—a Trypanosomal Disease of the Isthmus of Panama.**—*Jl. of Med. Research.* 1912. June. Vol. 26. No. 2. pp. 219–247.

Murrina, also known locally as Derrengadera and Morina de Cadera, is a disease of equines in the Panama Canal Zone. The cause is the *T. hippicum*. The author gives full details of the post-mortem findings in a number of natural and experimental cases in horses, mules, and ponies, and also the details of the lesions found in a number of monkeys, dogs, racoons, rabbits, guinea-pigs, mice and rats. Further details are also given regarding the histology of the lesions.

The following is an extract of the author's summary.

Murrina is an intoxication resulting in cellular degeneration and necrosis. The continuity of the endothelium is injured or destroyed and as a consequence effusion, oedema, and terminal ecchymoses arise. The toxic agents elicit certain reactions in the host: namely, lymphocytosis, auto-haemagglutination, phagocytosis of erythrocytes and trypanosomes, hyperplasia of the spleen, bone marrow, and lymph nodes, and cellular exudations in the kidney, liver and elsewhere.

The degree of splenic hyperplasia varies inversely with the size of the infected animal, being greatest in the smaller rodents, and is directly proportional to the anaemia and the degree of infection. The hepatic necroses and the collections of lymphoid cells in the liver and kidneys are of special interest. There are two types of necrosis: First, the large, central zone areas, in which the hepatic cells have undergone a hyaline, granular or fatty change, and in which there is usually no leucocytic exudation. Second, the smaller areas found in the intermediate and peripheral zones in which there is a polymorphonuclear and mononuclear leucocytic exudation replacing the parenchyma. Each type of necrosis suggests a different etiological factor. Again, in this infection there is a very marked lymphocytosis and in many of the areas of necrosis the cells may be entirely lymphoid in type or preponderatingly so. This requires further investigation.

The collection of cells in the liver and similar collections in the kidneys are of the polyblast and lymphocyte type, and they can be seen in many locations emerging from the arterioles and capillaries, having come originally from the spleen, in which organ they can be seen on the peripheries of the Malpighian bodies. These cells probably come from the lymph nodes and bone marrow as well. It would appear that the toxic agents in this infection specifically attract and stimulate the production of cells of the mononuclear type.

The cellular collections in the liver necroses are phagocytic in character and represent efforts to remove normal or degenerate hepatic cells.

The blood destruction and stimulation of the blood-forming organs leads to hyperplasia of the yellow bone marrow and to an interesting picture in the spleen of the coati, guinea-pig, monkey, rat and mouse. The spleens of these animals were found normally to contain a few megakaryocytes and nucleated red cells. Eosino-

philes were absent. Yet, when these animals were infected with *T. hippicum* the spleen, particularly that of the coati, took on to an intense degree the picture of red bone marrow, in that the megakaryocytes were greatly increased in number, and eosinophile myelocytes and polymorphonuclear leucocytes, as well as nucleated red cells, were seen. In this animal it would appear that the latent myelopoietic function of the spleen had been greatly augmented. The interstitial collections of cells in the kidneys of the racoon contained here and there some clumps of eosinophilic polymorphonuclear leucocytes, but no megakaryocytes.

In other mammals it was noted that when megakaryocytes normally were not seen in the spleen, the trypanosome infection did not provoke their appearance in any other location than in the marrow of the long bones.

In the experimentally produced disease in mules the autoagglutination of erythrocytes frequently appeared before the trypanosomes could be demonstrated in the peripheral blood.

The lesions in this trypanosomal disease conform with those of other trypanosome infections.

The lesions encountered in horses and mules were constant, though such features as oedema, emaciation, and ecchymoses varied with the stage of the disease. The gross lesions characteristic of the disease in equines are petechiae of the pleura, pericardium, nasal and conjunctival mucosae, peritoneum, renal cortex, and capsule of the spleen. There are haemorrhages of larger size in the endocardium, epicardium, and renal lymph nodes. Emaciation and anaemia are usually constant, though in the fulminating type of the disease the former may not be present. Effusions or localised areas of oedema of the sheath or hock, and particularly a longitudinal strip of the lower belly wall are seen. There is slight splenic enlargement and myeloid hyperplasia of the yellow marrow.

In smaller animals haemorrhages are not nearly so common; in fact, they are generally absent. Splenic enlargement becomes more noticeable and constant in the smaller animals. There was acute iridocyclitis in the dog, racoon, and monkey. Rabbits always presented dermatitis of the ears, and muzzle, and a marginal blepharitis. Ulceration of the scrotum was occasionally seen in guinea-pigs.

Histologically the lesions are distinctive. Nephritis was noted in horses and mules, but was different in type from that seen in the racoons and monkeys. The cellular changes in the liver of the guinea-pig are constant for that animal.

The features of the lesions in horses and mules are the agglutination of the red blood cells and their phagocytosis by endothelium and large mononuclear phagocytes in the liver, lymph nodes, and splenic sinuses; and hepatic necroses of two types, hyaline and inflammatory. Acute glomerulitis and acute haemorrhagic nephritis are very constant, as are the various petechiae and ecchymoses in which there may be polymorphonuclear or mononuclear leucocytic exudation. In the spleen the chief change noted is the very large amount of haemosiderin brought thither by large mononuclear phagocytes.

These changes occur with great rapidity in the small rodents and in these animals a great many trypanosomes are produced in proportion to the size of the animal in so short a time that they are rapidly overwhelmed. Trypanosomes are never as numerous in equines as in the small animals. Though equines are ultimately killed by the infection the lesions presented are, excepting the associated anaemia, very acute.

The histological changes in the smaller animals are generally more extensive, when similar in type, than those occurring in equines. The spleens show hyperplasia of all the lymphoid elements, some cellular necrosis and phagocytosis of pigment and of trypanosomes. In the kidneys of the smaller animals ecchymoses are uncommon, and the glomeruli are not involved. In monkeys and the racoon there is a very striking acute interstitial nephritis. The skin lesions and those of the iris show microscopic changes of the type characteristic of trypanosomiasis.

(19) Roudsky (D.). **Sur l'Immunité croisée entre le *Trypanosoma lewisi* et le *Tr. duttoni* renforcé.** [Cross-Immunity between *T. lewisi* and *T. duttoni*.]—*Compt. Rend. Soc. Biol.* 1912. April 26. Vol. 72. No. 14. pp. 609–611.

Although there are no distinct morphological differences between the *Trypanosoma lewisi* and the *T. duttoni* it is generally agreed that the two are distinct species, the *T. lewisi* being peculiar to the rat and the *T. duttoni* to the mouse.

The author has shown that each parasite can be acclimatised to the other host and this led him to investigate the question of cross immunity, or in other words whether a rat recovered from infection with the *T. lewisi* and hyperimmunised against that parasite would resist infection with the *T. duttoni*, and *vice versâ*.

Two experiments were made, and the results indicated that an animal that was immune to the *T. lewisi* was also immune to the *T. duttoni* and *vice versâ*. The immunity does not appear to be entirely phagocytory, although there is active phagocytosis. The destruction of the trypanosomes is not localised at the point of inoculation, and it proceeds so slowly that parasites injected into the peritoneum are able to multiply. Their passage into the blood stream is followed by extremely rapid destruction, and for this reason their presence there is quite the exception.

The *T. duttoni* does not appear to constitute a species distinct from the *T. lewisi*, which it closely resembles morphologically. The relative natural immunity possessed by the rat against this parasite is probably only one of chemical adaptation.

(20) Leger (A.) & Ringenbach (J.). **Sur la Spécificité de la Propriété trypanolytique des Sérums des Animaux trypanosomés. (Deuxième note.)** [The Specific Nature of the Trypanolytic Property of the Serum of Animals infected with Trypanosomiasis.]—*Compt. Rend. Soc. Biol.* 1912. Feb. 23. Vol. 72. No. 7. pp. 267–269.

In a previous note the authors have shown that the serum of animals affected with nagana and surra reacts not only with the homologous trypanosomes, but also with other trypanosomes that are considered to be allied to them, and that with others there is no reaction whatever.

In this new series of investigations experiments have been made with the *T. equinum*, *T. gambiense*, and *T. congolense*.

The technique of the experiments is as follows: The sera have been obtained from guinea-pigs and the viruses from infected mice. To 5 drops of freshly drawn serum are added one drop of citrate solution and one drop of blood from an infected mouse, the mixture being incubated for four hours.

The results obtained were similar to those previously obtained with the parasites of nagana and surra. It was found that the serum of an animal infected with the *T. gambiense* reacted with *T. rhodesiense*, and that of an animal infected with the *T. congolense* with the *T. dimorphon*.

It was observed that the serum of a guinea-pig infected with the *T. pecaudi* was not trypanolytic for any heterologous trypanosome. Although the *T. equinum* differs morphologically from the *T. evansi* the experiments revealed a relationship existing between the two.

This result agrees with the observations originated by Werbitzki, who has demonstrated the possibility of obtaining experimentally a transmission from a centrosomic to an acentrosomic variety of trypanosome.

In support of their view as to the value of their method of procedure the authors state that the serum of animals infected with the *T. brucei* (Zululand) at the Pasteur Institute proved trypanolytic for the same virus preserved at the Liverpool School of Tropical Medicine.

MISCELLANEOUS.

(21) Nattan-Larrier (L.). **La Coloration des Leishmania dans les Coupes.** [The Staining of Leishmania in Sections.]—*Compt. Rend. Soc. Biol.* 1912. March 22. Vol. 72. No. 11. pp. 436–438.

The pieces of tissue should not exceed 8 by 4 by 3 mm. They may be fixed in any of the following ways: Acetic sublimate or saturated solution of sublimate, which are probably the best. Alcohol of increasing strength, commencing with 70 per cent. and finishing with 90 per cent. Alcohol fixation may be modified by immersing the pieces of tissue in 2 per cent. formol for three or four hours beforehand.

The following three methods of staining have been employed by the author.

Carbol thionin.—The specimen is stained with carbol thionin for half an hour. It is then washed in distilled water, dehydrated with absolute alcohol, differentiated slowly with clove oil followed by absolute alcohol, and finally clarified with xylol.

The nucleus and centrosome of the Leishmania are stained dark blue, and stand out in contrast to the protoplasm which is faintly tinted blue. Parasites which are extracellular have their outlines well stained.

Kernschwarz and carbol thionin.—Stain with Kernschwarz for a quarter of an hour. Wash thoroughly in distilled water. Stain for half an hour with carbol thionin wash and dehydrate. Differentiate with oil of cloves followed by absolute alcohol until

only the nuclei remain stained. The nucleus and centrosome are stained a greenish-grey, the cytoplasm blue. The protoplasm of the host cells is greyish.

Alum carmine and carbol thionin.—Stain for 24 hours with alum carmine. Wash in distilled water and stain for half an hour with carbol thionin. Differentiate with oil of cloves until the section acquires a reddish-violet fluorescence, and a rapid inspection under the microscope shows that the protoplasm is pink. Dehydrate rapidly in absolute alcohol and clarify in xylol.

(22) HINDLE (E.). **Attempts to transmit "Fowl Pest" [Fowl Plague] by *Argas persicus*.—*Bull. Soc. Path. Exot.* 1912. March. Vol. 5. No. 3. pp. 165–167.**

Ticks fed upon infected birds at the height of the disease were unable to infect fresh birds by biting. The virus persists in the digestive tube of the tick for 9 days, but does not pass through the wall. It is not found in the coelom, nor in the coxal liquid.

The intestinal contents are not infective after 14 days. The ticks were kept at 22-28° C.

(23) CAROUGEAU. **Étude générale de l'Osteomalacie chez le Cheval, particulièrement en Madagascar.** [Osteomalacia in the Horse, and especially as observed in Madagascar.]—*Rev. Gén. de Méd. Vétér.* 1912. Jan. 1. Vol. 19. No. 217. pp. 1–19; and Jan. 15. No. 218. pp. 65–92.

Osteomalacia is in the opinion of the author an infectious disease and is characterised essentially by a progressive demineralisation of the bones.

Clinically the disease is recognised by lameness, pathognomic swelling of the bones of the head and jaws, detachment of ligaments and tendons from the bones, fractures, and frequently considerable wasting.

The lesions, which principally involve the osseous tissue and the bone marrow, are in the nature of a generalised osteomyelitis.

The author states that the disease termed osteomalacia occurring in the horse is entirely different from the conditions grouped under the same name occurring in other species of animals. The name is a misnomer since in the horse death occurs before the bones have become really soft.

The author gives a brief review of the literature on the subject and also mentions the occurrence of the disease in different parts of the world which in temperate countries is sporadic, and in hot countries endemic and epidemic.

The disease appears to have been observed in South Africa for the first time in 1885, and since then a number of observations have been made. Johannesburg appears to be a centre of the disease. The author has not been able to find any records of the disease occurring in Madagascar previous to the conquest, but after the introduction of horses and mules from different parts of the world in 1895 and 1897 the disease made its appearance. It would appear that the Madagascan horses were immune.

The author has seen cases in animals of all ages, the majority occurring in four- or five-year-olds. Cases occur at all altitudes,

in males and females, animals well-fed and ill-fed, and in animals at grass or in the stable.

Animals have been followed for periods of years, but the author has not been able to convince himself that the disease is hereditary. Animals that are used too young are more likely to be attacked than others, and certain races are less resistant than others.

The disease appears to persist in certain places, and this suggests the possibility that it is contagious.

The author's investigations have been directed towards verifying the contagious nature of the disease, studying the pathological anatomy of the disease, and attempting treatment.

In his search for the causal organism the author has not arrived at any definite result. Material has been taken from the bone marrow, synovia, and blood, and a number of ordinary and special media have been employed in attempts to cultivate it but without result.

A variety of organisms have been cultivated from such materials taken with aseptic precautions from animals either living or just dead. The majority of the cultures remained sterile or yielded a small micrococcus which appears "special" to Carougeau.

This organism is very small and frequently occurs in the form of diplococci. Cultures are very meagre, there being a scarcely perceptible growth on agar and only a faint opalescence in broth.

Abundant growths have never been obtained and subcultures have always proved sterile.

Microscopic examination of various materials has not yielded any interesting results.

Blood and materials derived from lesions have been used for inoculation experiments.

1. Horse.—Horses have been inoculated with blood, bone marrow, and bone ground up with sterile broth and filtered, but the results have been negative in every case.

Contact experiments have also failed to transmit the disease to healthy horses.

One experiment was made to transmit the disease to an ox by inoculating it subcutaneously with bone marrow. This also failed. Similarly, experiments with goats, sheep, dogs, rabbits, guinea-pigs, and, with one possible exception, pigs have also proved negative. The author's opinion that the disease is infectious is based upon the following considerations:

The occurrence of the disease under a variety of conditions of feeding, climate, and geological formations compels one to suppose that none of these conditions has a preponderating influence on the causation of the disease. On the other hand the repeated occurrence of the disease in certain places and certain stables suggests that it is of an infective nature. This view is supported by the nature of the lesions, which are those of a chronic irritation.

There is no doubt that defective feeding, inattention to hygiene and so on are contributing causes owing to the decrease of resistance occasioned, but in the author's view there is some essential factor.

Examination of the lesions shows that there is every evidence of active irritation; the bones are not simply decalcified. The inflammatory process is evidenced by the resorption of layers of osseous tissue, the formation of osteoid tissue, the production of a large amount of vascular connective tissue, and finally the nature of lesions in the marrow.

In the early stages it is the bone marrow that is principally involved; the disease is therefore an osteomyelitis which subsequently extends to the osseous tissue and joints.

The theory of infection also explains the digestive disorders always met with in cases of osteomalacia. There is always an unusually excessive elimination of phosphoric acid and calcium, but the quantity eliminated varies from time to time during the course of the disease, being generally greater in the early stages and in many cases nil in the last stages.

The author is of opinion that osteomalacia and rickets are two distinct conditions in the horse.

The lesions involve the bones and the joints and are in the nature of a generalised rarifying ostitis. All the other lesions found in cases of the disease are due to anaemia or general cachexia; there are never generalised lesions. Lesions never occur in the internal organs, save such as are of a secondary nature.

Any of the bones may be affected and the nature of the change is the same in all cases. The bones become lighter, and swollen, and lose their hard compact structure, the bone being converted into a porous sponge-like material which can be easily broken with the fingers. The long bones are less seriously affected than the others, and it is the bones of the head and the jaws that show the most marked alterations.

Joint lesions are constant and are generally more severe in the upper joints of the limbs than the lower ones.

Fractures occur commonly.

The medullary canal is always enlarged and the marrow has a pronounced red colour, although at the centre of the shaft of long bones it may be yellow. Even here there are often haemorrhages. In some cases the marrow resembles spleen pulp. The spaces in the cancellous tissue are enlarged.

Analysis of the bones shows that there is a diminution in the proportion of mineral matter and an increase in the animal matter, the variation depending upon the stage of the disease.

Microscopically the lesion is found to be the replacement of bony tissue by vascular connective tissue. This process may occur in any of the bones, but it is more marked in bones of fibrous origin than in those of cartilaginous origin.

The periosteum is found to be thickened, and beneath this the bone tissue becomes more and more rarified the deeper one examines it from the surface.

Lesions occur in the bone marrow in every case and at all stages of the disease. There is an absence of fat-containing cells and a multiplication of the medullary cells associated with extravasation of blood. Masses of red or yellowish blood pigment may be seen at places. In the later stages there is always some formation of fibrous tissue.

Previous to the deformation of the bones the symptoms presented are those that are to be expected in a disease of this kind.

As already mentioned, fractures commonly occur and it is not uncommon for ligaments and tendons to become detached from the bones even in the early stages of the disease.

Examination of the urine shows that the excretion of phosphoric acid is excessive. Whereas the urine of the horse normally contains about 40-80 centigrammes of phosphoric acid per day the author has found as much as 12·5 grammes per litre in a case of osteomalacia. The quantity excreted varies from case to case and from day to day in the same case.

The alkalinity of the blood is decreased, but in the case of recovery this returns to the normal. The number of red corpuscles may fall as low as 2,500,000 and there is also a decrease in the amount of haemoglobin. The leucocytes increase in number, but the differential counts vary greatly.

The course of the disease is slowly progressive, although at times there may be exacerbations which suddenly render an animal unfit for any work. Recovery is possible even when there are advanced lesions, but such recoveries are exceptional.

Treatment.—Attention to hygiene is of great value in the treatment of the disease. Animals should be left at rest at pasture.

Adrenalin appears to have a beneficial effect, but it proves too costly to be used very extensively.

Lime and magnesium salts have been used with good effect, as also have arsenic and mercury, the latter being administered intramuscularly in the form of calomel.

The author suggests that anhydro-oxymethylene-diphosphoric acid may prove to be a valuable remedy though he has not actually tried it.

24) Siegel (J.). **Einige ergänzende Bemerkungen zum Nachweis der Cytorrhyctescoccen bei Maul- und Klauenseuche.** [Supplementary Observations regarding the *Cytorhyctes cocci* in Foot and Mouth Disease.]—*Berlin. Tierärzt. Wochenschr.* 1912. Jan. 11. Vol. 28. No. 2. pp. 27–29.

The author has been able to find the cocci in smears taken from the heart muscle of four calves. In one case there was severe endocarditis with the formation of lesions on the valves of the right side, and in another very marked myocarditis, involving principally the left side and the apex. The author has seen lesions involving the valves of the heart in a pig inoculated experimentally with a pure culture, the inoculation being intraperitoneal. Pure cultures were obtained from two of the hearts.

Siegel's observations show that the infectivity of the blood and the number of cocci present in it run parallel.

Very good results have been obtained in the demonstration of the cocci in sections by staining with carbol-fuchsin, diluted three times with water, for half an hour and then washing in water, dehydrating with alcohol and mounting in balsam. The method is more rapid than that previously advised of staining with haematoxylin and methylene blue.

In sections of an unruptured lesion preserved in formalin immediately after the slaughter of the animal practically every cell of the inflamed connective tissue contains a group of the cocci. These generally lie close to the nucleus and are surrounded by a clear zone. The author retracts his previously expressed view that the cocci are actually in the nucleus.

The cocci vary greatly in size, but the majority are very minute.

(25) Uganda Protectorate. **Annual Report of the Veterinary Department for the Year 1911–1912.** [Abstracted from the *Ann. Rept. of Dept. of Agriculture for Year ending March* 31, 1912. pp. 18–28. (Entebbe : Govt. Printers).]

East Coast Fever.—The disease is highly endemic over all the districts to the west of the Nile, with the exception of the northern and central counties of Ankole.

In the Eastern Province East Coast Fever is endemic in the Busoga, Lango, and the greater part of the Mbale Districts.

In the Kumi District it is probable that the disease is not highly endemic, and the north eastern portion of the District is known to be free.

It is probable that Karamoja, which would appear to be one of the most heavily stocked districts in the Protectorate as regards cattle and sheep, is also a non-endemic area.

Rinderpest is still confined to the Eastern and Nile Provinces.

Trypanosomiasis.—Losses during the year have been exceptionally heavy. This has been most marked in Bulamwezi where a mortality of over 50 per cent. is reported in some herds. The responsible trypanosome appears to be the *T. pecorum.*

Cases have been reported of a disease closely resembling black quarter in the symptoms presented and the post-mortem appearances. Two cases were met with in which microfilariae were present in the blood of cattle.

(26) Todd (J. L.) & Wolbach (S. B.). **Parasitic Protozoa from the Gambia.** [Second Report of the Expedition of the Liverpool School of Tropical Medicine to the Gambia, 1911.]—*Jl. of Med. Research.* 1912. June. Vol. 26. No. 2. pp. 195–218.

This paper contains the results obtained by the examination of a small number of Gambian animals for parasitic protozoa, the observations being made as opportunity offered while investigations in connection with human trypanosomiasis were being carried out.

The authors state that possibly some of the parasites described are in the nature of post-mortem invaders, in view of the fact that in many cases the animals or birds suffered from wounds and some time elapsed before the examination was made; every precaution was taken, however, to prevent this post-mortem invasion.

Cattle and Horses.—

Trypanosomes.—In the blood of an Ayrshire bull, which was in a moribund condition when seen, large numbers of trypanosomes were found. There were two distinct types present. The

larger parasites had a free flagellum, and the smaller had not. The larger trypanosomes measured 20-25 microns in length, and the smaller 8-11. In both types the centrosome was in many instances terminal.

Similar trypanosomes were found in the blood of native horses.

Antelope.—

Spirochaetes.—A few spirochaetes were found in a specimen of stained blood. They varied considerably in size. The most numerous were short and somewhat thick, measuring 5-7·5 microns in length, but some were seen even shorter than this. The protoplasm was homogeneous or showed a single small clear area. They tapered towards each end slightly, but terminated bluntly.

The other type of spirochaete found was of about the same length but more slender.

Trypanosomes.—A few trypanosomes were present and the majority of these were of the "tadpole" type, measuring about 13 microns. There was also a larger form present measuring 15·5 microns by 3·7.

Rats inoculated with this parasite did not become infected.

Piroplasms.—Red corpuscles containing piroplasms occurred in almost every field of blood smears made from this animal.

The majority of the parasites were rounded in shape and contained a single mass of chromatin which was usually crescentic in form. Bacillary forms were found, but multiple infection of the same cell and bigeminal forms were rarely encountered.

The parasite was a small one, the round form averaging about 1 micron and the bacillary forms 2-2·5.

Granules of chromatin, which resembled the *Anaplasma marginale*, were found either in or at the periphery of some of the red cells. In the event of this proving to be a new species the name *Theileria hippotragi* is suggested.

There were no cattle in the forest where this antelope was shot but there were considerable herds at no great distance. None of the local chiefs had ever heard of redwater.

Rats.—

Trypanosomes.—Trypanosomes identical in appearance with the *T. lewisi* were found in the blood of rats caught in widely separated localities.

Birds.—

A leucocytozoon was found in the blood of a vulture.

This parasite measured about 7·5 by 3·7 microns. The cytoplasm was coarsely alveolar and stained deep blue. In many of the parasites there was a vacuole like area extending across one end. The nucleus was a loose mass of granular material lying near the middle point of the parasite, and in a few cases towards one end. No free parasites were observed, and all varieties except the small mononuclears and mast cells were found to be invaded, the majority being in large mononuclears. The name *Leucocytogregarina neophrontis* is suggested.

Hornbill.—Trypanosomes of two types were found in the blood of a hornbill.

The larger parasite measured about 40 microns. Its cytoplasm stained deeply and showed distinct myonemes.

The smaller parasite measured 26 microns. Myonemes were not so distinct as in the preceding. The centrosome was large and stained deeply, and was always placed about the centre of a curious prolongation of the posterior end of the body.

Doves.—Trypanosomes were found in the blood of two doves. The parasites were similar in morphology to the larger one occurring in the hornbill, but the centrosome was larger and the parasite measured 43 microns.

Two types of trypanosomes were found in the blood of bush fowl. The parasites were large, measuring 56·6 and 60·7 microns. The shorter of the two was provided with a blunt posterior extremity and the larger with a sharp one.

Trichomonads found in the blood of a bush fowl were probably post-mortem invaders, the shot wounds which killed the bird having penetrated the alimentary canal.

Leucocytozoa.—These parasites were found in the blood of four bush fowls. Most of the parasites were the large "adult" forms, the dark-staining females being about five times as numerous as the lighter coloured males. Small "young" forms were found to be very scanty in every case.

The parasites varied greatly in size, but the average size was 19-21 microns in length by 6-7 in width. The smallest measured ·5-2·5 microns.

The commonest form of the parasite with its host cell constituted a spindle-shaped body measuring 30-50 microns in length by 6-13 in width, the cytoplasm of the host-cell forming a kind of sheath. None of the cells containing parasites possessed any haemoglobin, nor were the parasites found in large mononuclear leucocytes.

It was impossible to determine with certainty the nature of the host cell, but the nuclei were like those of small mononuclears. Similar nuclei occur however in erythroblastic myelocytes.

The adult female parasites possessed a cytoplasm staining a deep blue. Its texture was coarsely alveolar. The bodies lying within the female parasites which stained like chromatin consisted of two large masses and an indefinite number of smaller granules. The larger of the two large masses was an oval or irregular mass of granules, and the smaller more compact and stained more deeply.

The line which was described as one of the nuclear bodies of a leucocytozoon in the blood of an African hawk was not present in these preparations.

The texture of the protoplasm of the male parasites was finer than that of the females, and it stained less densely. The larger chromatin mass was larger and less compact than in the female parasites, and the smaller mass was less frequently seen, but its relations were the same. The small forms had a deeply staining cytoplasm and one or more chromatin bodies.

Parasites of the Halteridium type were found in a number of different species and an inspection of the specimens suggested that there may be more than one species of halteridium.

Among the parasites found in the blood of reptiles were trypanosomes, haemocytozoa, and haemogregarines.

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